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8976

CONTENTS

No. 1, May, 1932

[Issued April 4, 1932.]

Page.

TANCHICO, SIMEONA S., and AUGUSTUS P. WEST. An aldehyde rosin oil from Philippine pine trees (Pinus insularis Endlicher) 1

CRUZ, AURELIO O., AUGUSTUS P. WEST, and VICENTE B. ARAGON. Composition of Philippine rice oil (ramai variety) 5

CRUZ, AURELIO O., and AUGUSTUS P. WEST. Composition of Philippine talisay oil from the seeds of Terminalia catappa Linnæus 13

ALEXANDER, CHARLES P. New or little-known Tipulidæ from the Philippines (Diptera), XIV 21
Three plates.

KUWANA, INOKICHI. Two new conifer-infesting scale insects from Japan 51
Three text figures.

KUWANA, INOKICHI. The genus Aclerda in Japan, including Formosa 57
Four text figures.

TAKAHASHI, RYOICHI. Additions to the aphid fauna of Formosa (Hemiptera) 69
Two text figures.

CRUZ, AURELIO O., and AUGUSTUS P. WEST. Composition of Philippine soy beans and soy-bean oil 77

AFRICA, CANDIDO M. Studies on experimental creeping eruption in the Philippines 89
Five plates and one text figure.

HIRANO, H. Study on a Philippine strain of Leptospira icterohæmorrhagiæ 103
One text figure.

TUBANGUI, MARCOS A. Observations on the possible transmission of surra by the land leech Hæmadipsa zeylanica 115
One plate.

No. 2, June, 1932

[Issued May 7, 1932.]

FAJARDO, T. G., and JOAQUIN MARAÑON. The mosaic disease of sincamas, Pachyrrhizus erosus (Linnæus) Urban 129
Six plates.

Page.

PANSHIN, ALEXIS J. An anatomical study of the woods of the Philippine mangrove swamps 143

Twenty-four plates.

PALO, MACARIO A. Anthracnose and important insect pests of the mango in the Philippines, with a report on blossom-spraying experiments 209

Eight plates.

TIRONA, MARIANO. The influence of the period of air drying on the strength of abacá fiber 237

TIRONA, MARIANO. The variability of tensile strength of commercial abacá fibers of the same origin in the pseudostem 243

HUMPHREY, C. J. Two conveniences for phytopathological work in the Tropics 257

Two plates.

RUSSELL, PAUL F. Avian malaria studies, IV. Hæmoproteus and Plasmodium in birds of Luzon, Philippine Islands 263

RUSSELL, PAUL F. Avian malaria studies, V. Plasmodium capistrani sp. nov., an avian malaria parasite in the Philippines 269

Two plates and one text figure.

No. 3, July, 1932

[Issued June 24, 1932.]

RUSSELL, PAUL F., and AUGUSTUS P. WEST. Charcoal as a diluent for Paris green in the destruction of anopheles larvæ; larvicide studies 291

One plate.

BAENS, LUZ, F. M. YENKO, AUGUSTUS P. WEST, and H. M. CURRAN. Composition of Philippine woods, III. Balobo, alupág, banaibanai, dulit, and pine 299

Two plates.

BORCHMANN, FRITZ. Die Alleculiden-Fauna der Philippinen 305

BORCHMANN, FRITZ. Erster Nachtrag zu die Lagriiden-Fauna der Philippinen 383

UMALI, AGUSTIN F. The Japanese beam trawl used in Philippine waters 389

Five plates and nine text figures.

TALAVERA, FLORENCIO. The fisheries of Lake Sampaloc, San Pablo, Laguna Province, Luzon 411

Four plates and nine text figures.

TALAVERA, FLORENCIO, and HERACLIO R. MONTALBAN. Fishing appliances of Panay, Negros, and Cebu 429

Fifteen plates and thirteen text figures.

No. 4, August, 1932

[Issued July 16, 1932.]

Page.

KING, W. V. Three Philippine Anopheles of the funestus-minimus subgroup 485

Two plates and eight text figures.

GEE, N. GIST. More Philippine Islands fresh-water sponges 525

Five text figures.

WEST, AUGUSTUS P., and PAUL F. RUSSELL. Paris green partially adsorbed on charcoal as a larvicide for anopheles mosquitoes; larvicide studies, II 545

Two plates.

MARAÑON, JOAQUIN, and JOSÉ K. SANTOS. Morphological and chemical studies on the seeds of Erythrina variegata var. orientalis (Linnæus) Merrill 563

Six plates.

HODGE-SMITH, T. Obsidianites in the Philippine Islands 581

Two plates.

YUTUC, LOPE M. Experiments on the transmission of surra by means of the dog hookworm, Ancylostoma caninum 589

ALEXANDER, CHARLES P. New or little-known Tipulidæ from the Philippines (Diptera), XV 597

Three plates.

INDEX 639

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THE PHILIPPINE JOURNAL OF SCIENCE

VOL. 48 MAY, 1932 No. 1

AN ALDEHYDE ROSIN OIL FROM PHILIPPINE PINE TREES (PINUS INSULARIS ENDLICHER)

By SIMEONA S. TANCHICO and AUGUSTUS P. WEST
Of the Bureau of Science, Manila

The Benguet pine (*Pinus insularis* Endlicher) grows extensively in the mountain regions of northern Luzon. Some months ago the resin content of Benguet pine was investigated[1] and the results showed that it yields turpentine and rosin of high grade. The rosin is obtained in the usual manner by steam distilling the pine resin; the turpentine passes over into the distillate leaving the crude rosin behind.

Experiments[2] have shown that the crude amorphous rosin may be converted by solvents into a crystallized form. When distilled the amorphous rosin is changed into a transparent glassy variety.

Recently we distilled the crude rosin in a current of alcohol. From the distillate we obtained an oil boiling at 80° to 81° C. This oil appears to consist mostly of an aldehyde.

EXPERIMENTAL PROCEDURE

The pine resin used in this investigation was collected by Ranger P. D. Esguerra, of the Philippine Bureau of Forestry. The resin was steam distilled to separate the turpentine, which passed over into the distillate, from the rosin which remained behind as the residue.

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[1] Santos, I. de, A. P. West, and J. Fontanoza, Philip. Journn. Sci. 45 (1931) 233, 383.

[2] Tanchico, S. S., A. P. West, and P. D. Esguerra, Philip. Journ. Sci. (in press).

Samples of commercial rosin, as ordinarily prepared, may give melting points that vary considerably. This is due to the fact that commercial rosin often contains varying amounts of oily substances, which are not removed during the steam distillation of the pine resin. The rosin that remains as a residue from steam distillation is not very hard and brittle. In a tropical climate it displays a tendency to soften and gradually flow due to the oil contained in it.

The crude rosin, obtained as a residue in distilling the pine-tree resin, was dissolved in alcohol and filtered to remove impurities such as twigs and chips of wood. The alcoholic solution of rosin was distilled with a current of alcohol vapor running through it. The distillate was found to consist of an alcoholic solution of light rosin oil. This light rosin oil appears to be the substance which causes the characteristic odor of Philippine rosin. The distillation was continued until the distillate, when treated with water, no longer gave a milky solution, thus indicating that most of the light rosin oil was removed from the rosin. To the alcoholic distillate, thus obtained, a considerable quantity of water was added to dissolve the alcohol. This produced a milky solution which was then extracted with ether. The ether extract was dehydrated first with calcium chloride and later with sodium sulphate, after which it was distilled to remove the ether. The residue consisting largely of the light rosin oil was then fractionated. The results are recorded in Table 1.

TABLE 1.—*Distillation of Benguet aldehyde rosin oil. (Amount of oil distilled 432 cubic centimeters.)*

Fraction.		Amount obtained.		Refractive index, $N\frac{30^\circ\text{ C.}}{D}$.	Specific gravity, $d\frac{30^\circ}{4^\circ}$ C.	Optical rotation $A\frac{30^\circ\text{ C.}}{D}$ (100 millimeter tube).
No.	Temperature.					
	°*C.*	*g.*	*Per cent.*			*Degrees.*
1	Below 79 cc.	56				
2	Above 79–80	103.55	23.96	1.3665	0.8218	+1.28
3	Above 80–82	92.7	21.45	1.3638	0.8279	+0.14
4	Above 82–86	21.0	4.86	1.3630	0.8403	−0.2
5	Above 86–95	15.2	3.51	1.3618	0.8435	−0.2
6	Residue	65.9	15.25	1.3605	0.8793	−0.2

The first fraction (Table 1) contains alcohol and ether and the residue some high boiling substances. The first fraction and the residue were discarded and the intermediate fractions

(Nos. 2 to 5) redistilled. After fractionating several times a fraction amounting to about 75 cubic centimeters was obtained.

This fraction had a fairly constant boiling point of 80° to 81° C. Other constants of this fraction were as follows:

Specific gravity $\left(d\,\frac{30^\circ}{4^\circ}\mathrm{C.}\right)$ 0.8382

Specific rotation $\left(A\,\frac{30^\circ\,\mathrm{C.}}{D}\right)$ + 1.32

Refractive index $\left(N\,\frac{30^\circ\,\mathrm{C.}}{D}\right)$ 1.3636

This oil gave the usual tests for double bonds such as decolorizing a bromine solution and an alkaline solution of potassium permanganate.

The oil gave very decided tests for aldehydes as follows:

A red color with Schiff's reagent within a time limit of two minutes.
Orange color with a glacial acetic acid solution of benzidine.
Reduced Fehling's solution giving cuprous oxide precipitate.
Gave positive test with Tollen's reagent.

From 12.5 grams of oil there was obtained 9.9 grams of the aldehyde sodium bisulphite compound.

When mixed with water a milky solution is obtained but the oil is soluble in all the common organic solvents.

The yield of this aldehyde oil (80° to 81° C.) from the crude rosin was found to be about 4 per cent.

We expect to continue this investigation when more of this aldehyde oil is available.

We wish to thank Mr. Arthur F. Fischer, director, Bureau of Forestry, and Mr. P. D. Esguerra, Bureau of Forestry, for their assistance in this investigation.

SUMMARY

Rosin was prepared from the resin of Benguet pine trees (*Pinus insularis* Endlicher). This rosin yields a colorless, light, aldehyde rosin oil. The yield is about 4 per cent.

This aldehyde oil boils at from 80° to 81° C. It gives a milky solution with water but is soluble in the common organic solvents.

COMPOSITION OF PHILIPPINE RICE OIL (RAMAI VARIETY)

By AURELIO O. CRUZ and AUGUSTUS P. WEST
Of the Bureau of Science, Manila

and

VICENTE B. ARAGON
Of the University of the Philippines, Manila

As rice is the staple diet of the inhabitants of the Far Eastern countries the rice bran (polishings) naturally accumulates in considerable quantities as a by-product. The bran comprises the seed coat and germ of the rice grain. It is now used for cattle food and to a slight extent for making tikitiki extract, which is employed in treating the disease known as beriberi.

Rice oil occurs in rice bran. Recently we analyzed Philippine rice oil made from bran of the ramai variety of rice. Our results showed that the oil consists principally of glycerides of oleic, linolic, and palmitic acids and is very similar in composition to kapok, cottonseed, and peanut oil. Rice oil has a bland, fatty taste and according to the composition is suitable for various purposes, such as making edible products and soap, for which cottonseed oil may be employed.

Some years ago the attempt was made to produce rice oil commercially but Lewkowitsch[1] states that the venture was not successful due, probably, to the low price of oils and fats which prevailed at that time.

When rice bran is stored the oil in the bran hydrolyzes very rapidly with the production of a considerable quantity of free fatty acids. According to Browne[2] this rapid decomposition of the oil is due to a fat-splitting enzyme. Experiments carried out by Browne indicated that the fat-splitting enzyme contained in the bran could be destroyed by heating the bran to a temperature of about 90° C. To prevent the formation of free acids in rice bran and the resulting rancidity Browne suggested heating the bran, immediately after milling, to a dry heat sufficient to destroy the enzyme, such as is done in the kiln-drying of certain seeds.

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[1] Chemical Technology and Analysis of Oils, Fats, and Waxes 2 (1922) 330.

[2] Journ. Am. Chem. Soc. 25 (1903) 948.

Ramai rice is a Philippine lowland variety which requires plenty of water for cultivation. Although it matures slowly it is the highest yielding variety in the Philippines. An account of the cultivation of ramai rice at the Central Luzon Agricultural School is given by Vicente Aragon.[3] At this school and in nearby localities it was found that this variety requires nearly two hundred days to mature. The highest yields are obtained by transplanting the rice. In ordinary irrigated land the yield may be more than 100 cavanes per hectare.

Ramai rice has rather large grains which have an average length of 7.74 millimeters; an average width of 3.47 millimeters; and an average thickness of 2.23 millimeters. As an edible product the ramai rice is not as tasty as some varieties of rice which have smaller grains.

EXPERIMENTAL PROCEDURE

This ramai rice was milled under the supervision of Vicente Aragon, of the College of Agriculture, University of the Philippines. The bran that was sufficiently fine to pass a 40-mesh sieve was extracted for two days with ether. The ether extract was then filtered and the filtrate distilled to remove the ether. The rice oil was then treated successively (warming, shaking, and filtering) with kieselguhr, suchar, and talcum powder. The brilliantly clear oil thus obtained had a rather dark brown color with a greenish tinge. The yield was about 18 per cent calculated on a moisture-free basis. The physical and chemical constants of this ramai variety of rice oil are given in Table 1.

TABLE 1.—*Physical and chemical constants of Philippine rice oil (ramai variety).*

Specific gravity at $\frac{30^\circ}{4^\circ}$ C.	0.9059
Refractive index at 30° C.	1.4662
Iodine number (Hanus)	99.3
Saponification value	185.9
Unsaponifiable matter (per cent)	4.02
Acid value	42.16
Saturated acids, determined (per cent)	20.71
Unsaturated acids plus unsaponifiable matter, determined (per cent)	72.71
Saturated acids, corrected (per cent)	19.95
Unsaturated acids, corrected (per cent)	69.68
Iodine number of unsaturated acids (determined)	124.7

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[3] Philip. Agr. 18 (1930) 535.

The saturated and unsaturated acids that occur as glycerides in Philippine rice oil (ramai) were separated by the lead-salt-ether method[4] in accordance with the suggestions of Baughman and Jamieson.[5] The results are recorded in Table 2.

TABLE 2.—*Separation of saturated acids from the unsaturated acids in Philippine rice oil (ramai variety) by the lead-salt-ether method.*

Experiment No.	Oil used.	Unsaturated acids.	Saturated acids.	Unsaturated acids (determined).	Saturated acids (determined).	Unsaturated acids (corrected).[a]	Saturated acids (corrected).
	g.	*g.*	*g.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	9.4675	6.9111	1.9570	73.00	[b]20.67	69.99	19.88
2	9.6694	7.0012	2.0052	72.41	[c]20.74	69.36	20.02
Mean				72.71	20.71	69.68	19.95

[a] Unsaturated acids (with unsaponifiable matter removed); iodine number (Hanus), 124.7.

[b] Iodine number (Hanus), 4.8.

[c] Iodine number (Hanus), 4.3.

The unsaturated acids separated from ramai rice oil by the lead-salt-ether method were treated with bromine and converted into their bromo-derivatives.[6] No ether-insoluble hexabromide was obtained, thus showing the absence of linolenic acid. The composition of the mixed unsaturated acids, which occur as glycerides in ramai rice oil, was calculated from the iodine number of the unsaturated acids. The results are recorded in Table 3. There are also included the calculated percentages of glycerides corresponding to these individual unsaturated acids.

TABLE 3.—*Percentage composition of the unsaturated acids of rice oil (ramai variety) and the glycerides corresponding to these acids.*

Acid.	Mixture of unsaturated acids.	Original oil.	Glycerides in original oil.
	Per cent.	*Per cent.*	*Per cent.*
Linolic	37.84	26.37	27.56
Oleic	62.16	43.31	45.26
Total	100.00	69.68	72.82

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[4] Lewkowitsch, J., Chemical Technology and Analysis of Oils, Fats, and Waxes 1 (1921) 556.

[5] Cotton Oil Press 6 (1922) 41. Journ. Am. Chem. Soc. 42 (1920) 2398.

[6] Lewkowitsch, J., Chemical Technology and Analysis of Oils, Fats, and Waxes 1 (1921) 585.

Saturated acids.—The saturated acids were separated from ramai rice oil by the lead-salt-ether method and esterified with methyl alcohol. The mixed acids were dissolved in methyl alcohol and saturated with dry hydrogen chloride gas. The mixture was then heated on a water bath (reflux) for fifteen hours, after which it was treated with water and the ester layer separated. The esters were dissolved in ether and the ethereal solution washed with sodium carbonate solution and afterwards with water. The ethereal solution was then dehydrated with anhydrous sodium sulphate, filtered, and the ether removed by distilling. The impure esters, which were yellow, were distilled under diminished pressure. A preliminary distillation at about 3.5 millimeters pressure was made. The esters were then redistilled at 5 millimeters pressure. Data on the distillation of the esters are given in Tables 4 and 5.

TABLE 4.—*First distillation of the methyl esters of the saturated acids; pressure, 3.5 millimeters; 111.2771 grams of esters distilled.*

Fraction—	Temperature.	Weight.
	°C.	g.
A	145–164	26.0452
B	164–169	24.1373
C	169–173	23.9360
D	173–180	18.8872
E	180–195	11.2864
F	195–215	3.8434
Residue		2.95
Total		111.0772

TABLE 5.—*Second distillation of the methyl esters of the saturated acids; pressure, 5 millimeters; 111.0772 grams of esters redistilled.*

Fractions.		Temperature.	Weight.
From first distillation.	Second distillation.		
		°C.	g.
A and B	1	175–177	11.3975
C	2	177–181	43.9698
D	3	181–184	29.7490
E and F	4	184–190	7.8825
Residue	5	190–195	5.8610
	6	195–210	5.3332
	7	210–225	4.7912
	Residue		2.07
Total			111.0542

In Table 6 are given the analyses of fractions obtained in the second distillation of methyl esters. From the data (Table 6) there were calculated the amounts of the individual acids corresponding to the methyl esters contained in the various fractions. The results are recorded in Table 7 and were calculated in accordance with the methods outlined by Baughman and Jamieson in their investigations of Hubbard squash-seed oil[7] and also American cottonseed oil.[8]

TABLE 6.—*Analyses of fractions obtained in the second distillation of the mixed methyl esters.*[a]

Fraction—	Iodine number.	Saponification value.	Mean molecular weight of mixed esters.	Composition of mixed esters.		Mean molecular weight of saturated esters.
				Saturated.	Unsaturated.	
				Per cent.	*Per cent.*	
1	1.10	208.3	269.3	0.93	99.07	269.1
2	1.79	206.3	271.9	1.51	98.49	271.7
3	3.98	205.2	273.4	3.35	96.65	272.7
4	7.33	201.4	278.6	6.18	93.82	277.4
5	11.12	197.7	283.8	9.37	90.63	282.6
6	14.15	186.9	300.2	11.92	88.08	300.8
7	7.94	159.6	351.5	6.69	93.31	358.7

[a] Calculated iodine number of unsaturated methyl esters was 118.7. Calculated saponification value of unsaturated methyl esters was 190.0.

TABLE 7.—*Saturated acids corresponding to methyl esters in each fraction.*

Fraction—	Acid.									
	Myristic.		Palmitic.		Stearic.		Arachidic.		Lignoceric.	
	Per cent.	*g.*	*Per cent.*	*g.*	*Per cent.*	*g.*	*Per cent.*	*g.*	*Per cent.*	*g.*
1	3.99	0.45	89.93	10.25						
2			88.72	39.01	4.69	2.06				
3			83.79	24.93	7.89	2.35				
4			66.40	5.23	22.67	1.79				
5			48.19	2.82	37.94	2.22				
6					76.45	4.08	7.53	0.40		
7							37.73	1.81	51.92	2.49
Residue[a]										1.99
Total		0.45		82.24		12.50		2.21		4.48

[a] Residue assumed to be methyl lignocerate.

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[7] Journ. Am. Chem. Soc. 42 (1920) 156.

[8] Op. cit. 1197.

In Table 8 is given the composition of the mixed saturated acids and the glycerides in the original sample of ramai rice oil corresponding to these acids.

TABLE 8.—*Saturated acids.*

Acid.	Mixture of saturated acids.[a]			Glycerides in original oil.
	Weight.	Composition.	Proportions in original oil.	
	g.	*Per cent.*	*Per cent.*	*Per cent.*
Myristic	0.45	0.44	0.09	0.10
Palmitic	82.24	80.72	16.10	16.89
Stearic	12.50	12.27	2.45	2.56
Arachidic	2.21	2.17	0.44	0.46
Lignoceric	4.48	4.40	0.87	0.90
Total	101.88	100.00	19.95	20.91

[a] When separated from rice oil the corrected percentage of saturated acids was 19.95.

The composition of ramai rice oil is given in Table 9. There are also included for comparison the analysis of Philippine hambas rice oil and the composition of a sample of rice oil analyzed by G. S. Jamieson.

TABLE 9.—*Composition of rice oil.*

Constituent.	Philippine rice oil (ramai variety).	Philippine rice oil (hambas variety)[a] analyzed by A. O. Cruz and A. P. West.	Rice oil[b] analyzed by G. S. Jamieson.
	Per cent.	*Per cent.*	*Per cent.*
Glycerides of:			
Unsaturated acids—			
Oleic	45.3	45.6	41.0
Linolic	27.6	27.7	36.7
Saturated acids—			
Myristic	0.10	0.2	0.3
Palmitic	16.9	17.3	12.3
Stearic	2.6	1.8	1.8
Arachidic	0.5	0.7	0.5
Lignoceric	0.90	0.7	0.4
Unsaponifiable matter	4.0	4.0	4.6
Total	97.9	98.0	97.6

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[a] Philip. Journ. Sci. (in press).

[b] This oil was an ether extract of rice bran made by C. E. F. Gersdorff, of the Bureau of Chemistry, Washington, Journ. Oil and Fat Indus. 3 (1926) 256.

TABLE 10.—*Comparison of Philippine rice oil (ramai) with other oils.*

Constituent.	Philippine oils.			American oils.	
	Philippine rice oil (ramai variety).	Kapok-seed oil.[a]	Peanut oil.[b]	Cottonseed oil.[c]	Peanut oil.[d]
Glycerides of:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Unsaturated acids—					
Oleic	45.3	49.8	53.9	35.2	52.9
Linolic	27.6	29.3	27.0	41.7	24.7
Saturated acids—					
Myristic	0.10	0.5	-----	0.3	-----
Palmitic	16.9	15.9	8.5	20.0	8.2
Stearic	2.6	2.3	3.6	2.0	6.2
Arachidic	0.5	0.8	3.4	0.6	4.0
Lignoceric	0.90	-----	2.4	-----	3.1
Unsaponifiable matter	4.0	0.8	0.3	-----	0.2
Total	97.9	99.4	99.1	99.8	99.3

[a] Philippine kapok-seed oil (*Ceiba pentandra* Gaertner) analyzed by A. O. Cruz and A. P. West, Philip. Journ. Sci. 46 (1931) 131.

[b] Philippine peanut oil (Valencia variety) analyzed by A. O. Cruz and A. P. West, Philip. Journ Sci. 46 (1931) 199.

[c] American cottonseed oil analyzed by G. S. Jamieson and W. F. Baughman, Journ. Am. Chem. Soc. 42 (1920) 1197.

[d] American peanut oil (Spanish type) analyzed by G. S. Jamieson, W. F. Baughman, and D. Brauns, Journ. Am. Chem. Soc. 43 (1921) 1372.

In Table 10 is given the composition of Philippine rice oil (ramai) compared with other Philippine and American oils. As shown by the data the rice oil is very similar in composition to kapok, cottonseed, and peanut oils. All of these oils consist principally of glycerides of oleic, linolic, and palmitic acids. They are suitable commercially for the various purposes for which cottonseed oil is employed; that is, the high-grade oils are useful for making edible products while the lower grades may be employed in soap making.

The commercial possibilities of rice oil would depend upon the current price and available supply of other oils; such as, coconut, cottonseed, etc. In case of a scarcity of these other oils the rice oil may become a commercial product of considerable importance.

A serious drawback to the commercial development of the rice-oil industry is that the oil is not easily expressed from the bran. Recently, we tried to express the oil from rice bran by using a hydraulic press of the coconut-oil mill of the Spencer, Kellogg Company. With a pressure of 4,000 pounds, which is commonly employed for expressing coconut oil, practically no oil was obtained from rice bran. To produce rice oil commercially it would seem that an extraction plant would be necessary.

We wish to thank Mr. H. Hellis, of the Spencer, Kellogg Company, who very kindly permitted us to use a hydraulic press.

SUMMARY

Rice oil is obtained from rice bran which comprises the seed coat and germ of the rice grain.

In this investigation we determined the composition of rice oil obtained from bran of the ramai variety of rice. Our results showed that this kind of rice oil consists principally of glycerides of oleic, linolic, and palmitic acids. Ramai rice oil is very similar in composition to kapok, cottonseed, and peanut oils. These oils are suitable commercially for the manufacture of edible products and soaps and for other purposes for which cottonseed oil may be employed.

To produce rice oil commercially it would seem that an extraction plant would be necessary.

Rice oil may be an additional source of income for the rice industry.

COMPOSITION OF PHILIPPINE TALISAY OIL FROM THE SEEDS OF TERMINALIA CATAPPA LINNÆUS

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The seeds of the plant known botanically as *Terminalia catappa* Linnæus have a kernel which is edible. The kernel yields an oil known locally as talisay oil. Recently we determined the composition of this oil and the results showed that it consists principally of glycerides of oleic, linolic, and palmitic acids. The oil is very similar in composition to kapok, cottonseed, and peanut oils and may be used for the same purposes for which cottonseed oil is employed—that is, the manufacture of edible fats, soaps, and other products.

In India, talisay oil is called Indian almond oil because it resembles oil of sweet almonds. Concerning Indian almond oil Watt[1] gives the following account:

> The kernels yield a valuable oil, similar to almond oil in flavour, odour, and specific gravity, but a little more deeply coloured; it deposits stearine on keeping. It possesses the advantage of not becoming rancid so readily as true almond oil, and if it could be produced cheaply would doubtless compete successfully with it. As the tree is abundant everywhere and the fruit could be doubtless obtained very cheaply, "Indian almond oil" appears to merit the attention of dealers. It was first brought prominently to notice by a Mr. A. T. Smith of Jessor, who in 1843 wrote to the Agr.-Horticultural Society of India an account of its properties and method of preparation. Oil, made experimentally by him, was expressed in the common native mill—a sort of pestle and mortar—from some fruit gathered during a few mornings from under the trees in the neighborhood. After a sufficient quantity had been gathered and allowed to dry in the sun for a few days, which facilitates breaking the nut, four coolies were set to work with small hammers, to separate the kernels from their shells. In four days they broke sufficient quantity for one mill, viz., 6 seers. This quantity put into the mill produced in three hours about 3 pucka seers of oil. Mr. Smith remarks that the actual pressing of the oil is of no consideration, since the value of the oil-cake, to feed pigs, etc., is sufficient to cover the expense, but the breaking of the nuts is a tedious and costly operation, and is a consideration requiring particular attention, with a view to its reduction, if manufacture of the oil on an extensive scale should be attempted. The product of the experiment, filtered through blotting paper, was of the colour of pale sherry, a circumstance which Mr. Smith explains is due to the rind being allowed to remain on the kernels. He concludes by remarking on the ornamental

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[1] A Dictionary of the Economic Products of India pt. 4, 6 (1893) 23.

nature and utility of the tree for many other purposes, and recommends that it should be more extensively planted. A sample of the oil thus prepared was submitted for examination to Doctor Mouat, who reported as follows:—"I have compared the specimen with a good muster of the ordinary European almond oil in my possession, and find that in taste, smell, and specific gravity, the former is very similar to the latter, but is deeper in colour, becomes turbid in keeping, and deposits a quantity of white stearic matter. For most ordinary purposes, medicinal and otherwise, the former, I think, might profitably be substituted for the latter in this country, and, if expressed with greater care and freed from every impurity, might become an article of commercial value and importance" (Journ. Agr.-Hort. Soc. Ind., ii.). Though easily made edible and pleasant in flavour, it appears to have been entirely neglected by the Natives, who are ignorant as to its existence.

Terminalia catappa is a tree which reaches a height of 25 meters. In the Philippines this species usually grows near the seashore and is distributed from northern Luzon to southern Mindanao. It is cultivated to some extent as a shade tree in and about Manila and many provincial towns.

EXPERIMENTAL PROCEDURE

The Philippine talisay seeds used in this investigation were kindly given us by Dr. Manuel Roxas, director of the Bureau of Plant Industry. The seeds were broken open and the kernels removed and ground in a mill. The pulp was then extracted with ether to obtain the talisay oil. The oil was purified by treating successively with 2 per cent kieselguhr, suchar, and talcum powder. This treatment removes vegetable fibers and colloidal matter and produces a clear oil which is slightly yellow. The yield of oil obtained from the kernel was about 52 per cent. Calculated on the weight of the whole seed the yield was only about 3 per cent.

The constants of this sample of talisay oil are given in Table 1.

TABLE 1.—*Physical and chemical constants of Philippine talisay oil.*

Specific gravity at $\frac{30^\circ}{4^\circ}$ C.	0.9046
Refractive index at 30° C.	1.4644
Iodine number (Hanus)	75.4
Saponification value	193.2
Unsaponifiable matter (per cent)	0.54
Acid value	2.5
Saturated acids, determined (per cent)	34.82
Unsaturated acids plus unsaponifiable matter, determined (per cent)	59.36
Saturated acids, corrected (per cent)	32.62
Unsaturated acids, corrected (per cent)	61.01
Iodine number of unsaturated acids	122.9

The saturated and unsaturated acids that occur as glycerides in Philippine talisay oil were separated by the lead-salt-ether method[2] in accordance with the suggestions of Baughman and Jamieson.[3] The results are recorded in Table 2.

TABLE 2.—*Separation of saturated acids from the unsaturated acids of talisay oil by the lead-salt-ether method.*

Experiment No.—	Oil used.	Unsaturated acids.	Saturated acids.	Unsaturated acids (determined).	Saturated acids (determined).	Unsaturated acids (corrected).[a]	Saturated acids (corrected).
	g.	*g.*	*g.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	10.0199	5.9397	3.4919	59.28	[b] 34.85	60.98	32.61
2	10.6200	6.3114	3.6941	59.43	[c] 34.78	61.04	32.63
Mean				59.36	34.82	61.01	32.62

[a] Unsaturated acids (unsaponifiable matter removed); iodine number (Hanus), 122.9.
[b] Iodine number (Hanus), 7.9.
[c] Iodine number (Hanus), 7.6.

The unsaturated acids separated from talisay oil by the lead-salt-ether method were treated with bromine and converted into their bromo-derivatives.[4] No ether-insoluble hexabromide was obtained, thus showing the absence of linolenic acid. The composition of the mixed unsaturated acids, which occur as glycerides in talisay oil was calculated from the iodine number of the unsaturated acids. The results are recorded in Table 3. There are also included the calculated percentages of glycerides corresponding to these individual unsaturated acids.

TABLE 3.—*Composition of the unsaturated acids of talisay oil and the glycerides corresponding to these acids.*

Acid.	Mixture of unsaturated acids.	Original oil.	Glycerides in original oil.
	Per cent.	*Per cent.*	*Per cent.*
Linolic	35.93	21.92	22.91
Oleic	64.07	39.09	40.85
Total	100.00	61.01	63.76

Saturated acids.—The saturated acids were separated from talisay oil by the lead-salt-ether method and esterified with methyl alcohol. The mixed acids were dissolved in methyl alcohol and saturated with dry hydrogen chloride gas. The mixture was then heated on a water bath (reflux) for fifteen hours after which it was treated with water and the ester layer separated. The esters were dissolved in ether and the ethereal solution washed with sodium carbonate solution and afterwards with water. The ethereal solution was then dehydrated with anhydrous sodium sulphate, filtered, and the ether removed by distilling. The impure esters which were yellow, were distilled under diminished pressure. A preliminary distillation at about 4 millimeters pressure was made. The esters were then redistilled at 4 millimeters pressure. Data on the distillation of the esters are given in Tables 4 and 5.

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[2] Lewkowitsch, J., Chemical Technology and Analysis of Oils, Fats, and Waxes 1 (1921) 556.

[3] Cotton Oil Press 6 (1922) 41. Journ. Chem. Soc. 42 (1920) 2398.

[4] Lewkowitsch, J., Chemical Technology and Analysis of Oils, Fats, and Waxes 1 (1921) 585.

TABLE 4.—*First distillation of the methyl esters of the saturated acids; pressure, 4 millimeters; 123.2236 grams of esters distilled.*

Fraction—	Temperature.	Weight.
	°C.	g.
A	165–178	19.4998
B	178–180	44.6762
C	180–182	22.1372
D	182–187	17.3757
E	187–208	16.6851
Residue		2.68
Total		123.0540

TABLE 5.—*Second distillation of the methyl esters of the saturated acids; pressure, 4 millimeters; 123.0540 grams of esters redistilled.*

Fractions.		Temperature.	Weight.
From first distillation.	Second distillation.		
		°C.	g.
A and B	1	165–173	13.8711
C	2	173–176	35.4190
D	3	176–178	27.9824
E	4	178–181	16.7412
Residue	5	181–187	13.8065
	6	187–192	7.4070
	7	192–214	5.6981
	Residue		2.07
Total			122.9953

In Table 6, are given the analyses of fractions obtained in the second distillation of methyl esters. From the data (Table

6), there were calculated the amounts of the individual acids corresponding to the methyl esters contained in the various fractions. The results are recorded in Table 7 and were calculated in accordance with the methods outlined by Baughman and Jamieson in their investigations of Hubbard squash-seed oil[5] and also American cottonseed oil.[6]

TABLE 6.—*Analyses of fractions obtained in the second distillation of the mixed methyl esters.*[a]

Fraction—	Iodine number.	Saponification value.	Mean molecular weight of mixed esters.	Composition of mixed esters.		Mean molecular weight of saturated esters.
				Saturated.	Unsaturated.	
				Per cent.	*Per cent.*	
1	5.0	208.9	268.5	95.73	4.27	267.4
2	6.4	207.6	270.2	94.53	5.47	268.9
3	11.5	205.6	272.9	90.18	9.82	270.6
4	22.2	201.5	278.4	81.04	18.96	274.7
5	35.0	198.1	283.2	70.11	29.89	278.4
6	48.4	190.2	295.0	58.67	41.33	294.6
7	44.1	188.1	298.2	62.34	37.66	300.2

[a] Calculated iodine number of unsaturated methyl esters was 117.1. Calculated saponification value of unsaturated methyl esters was 190.0.

TABLE 7.—*Saturated acids corresponding to methyl esters in each fraction.*

Fraction—	Acid.							
	Myristic.		Palmitic.		Stearic.		Arachidic.	
	Per cent.	*g.*	*Per cent.*	*g.*	*Per cent.*	*g.*	*Per cent.*	*g.*
1	9.31	1.29	81.40	11.29				
2	4.44	1.57	85.17	30.17				
3			84.60	23.67	0.91	0.25		
4			67.77	10.84	12.13	2.03		
5			47.25	6.52	19.33	2.67		
6			7.35	0.54	48.53	3.59		
7					55.38	3.16	4.05	0.23
Residue[a]								1.98
Total		2.86		83.03		11.70		2.21

[a] Residue assumed to be methyl arachidate.

In Table 8 is given the composition of the mixed saturated acids and the glycerides in the original sample of talisay oil corresponding to these acids.

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[5] Journ. Am. Chem. Soc. 42 (1920) 156.

[6] Op. cit. 1197.

TABLE 8.—*Saturated acids.*

Acid.	Mixture of saturated acids.			Glycerides in original oil.
	Weight.	Composition.	Proportions in original oil.	
	g.	*Per cent.*	*Per cent.*	*Per cent.*
Myristic	2.86	2.87	0.94	1.00
Palmitic	83.03	83.20	27.14	28.47
Stearic	11.70	11.72	3.82	3.99
Arachidic	2.21	2.21	0.72	0.75
Total	99.80	100.00	32.62	34.21

In Table 9 is given the composition of Philippine talisay oil compared with other Philippine and American oils. As shown by the data the talisay oil is very similar in composition to kapok, cottonseed, and peanut oils. All of these oils consist principally of glycerides of oleic, linolic, and palmitic acids. They are suitable commercially for the various purposes for which cottonseed oil is employed; that is, the high-grade oils are useful for making edible products while the lower grades may be employed in soap making.

TABLE 9.—*Comparison of Philippine talisay oil with other oils.*

Constituent.	Philippine oils.				American oil.
	Talisay oil.	Rice oil (hambas).[a]	Kapok oil.[b]	Peanut oil.[c]	Cottonseed oil.[d]
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Glycerides of:					
Unsaturated acids—					
Oleic	40.85	45.6	49.8	53.9	35.2
Linolic	22.91	27.7	29.3	27.0	41.7
Saturated acids—					
Myristic	1.00	0.2	0.5	----------	0.3
Palmitic	28.47	17.3	15.9	8.5	20.0
Stearic	3.99	1.8	2.3	3.6	2.0
Arachidic	0.75	0.7	0.8	3.4	0.6
Lignoceric	----------	0.7	----------	2.4	----------
Unsaponifiable matter	0.54	4.0	0.8	0.3	----------
Total	98.51	98.0	99.4	99.1	99.8

[a] Philippine rice oil (hambas variety), A. O. Cruz, A. P. West, and N. B. Mendiola, Philip. Journ. Sci. (in press).

[b] Philippine kapok oil (*Ceiba pentandra* Gaertner), A. O. Cruz and A. P. West, Philip. Journ. Sci. 46 (1931) 131.

[c] Philippine peanut oil (Valencia variety), A. O. Cruz and A. P. West, Philip. Journ. Sci. 46 (1931) 199.

[d] American cottonseed oil, G. S. Jamieson and W. F. Baughman, Journ. Am. Chem. Soc. 42 (1920) 1197.

NEW OR LITTLE-KNOWN TIPULIDÆ FROM THE PHILIPPINES (DIPTERA), XIV[1]

By CHARLES P. ALEXANDER

Of Amherst, Massachusetts

THREE PLATES

The crane flies described in this report are all from Mindanao, where they were taken in Surigao Province by Mr. A. C. Duyag, and in Davao district by Mr. C. F. Clagg. The collections made by Mr. Duyag are rich in species that were not found by Mr. Clagg in his intensive collecting in the La Lun Mountains and on Mount Apo. I am very greatly indebted to the collectors, and to Mr. Richard C. McGregor, for the continued interest in making known the rich tipulid fauna of the Philippines. The types are preserved in my collection.

TIPULINÆ

DOLICHOPEZA (NESOPEZA) ABDITA sp. nov. Plate 1, fig. 1; Plate 2, fig. 23.

General coloration dark brown; antennæ black, the basal portion of the first segment obscure yellow; legs dark brown, the outer tarsal segments white; wings with a strong blackish suffusion; Rs long; cell 2d A wide.

Male.—Length, about 10 millimeters; wing, 12.

Frontal prolongation of head obscure yellow; palpi black. Antennæ of moderate length, if bent backward extending about to base of abdomen; basal portion of scape yellow, the remainder of antennæ black; flagellar segments subcylindrical, with numerous coarse verticils. Head brown; anterior vertex broad.

Mesonotum chiefly brown, the præscutum indistinctly striped with darker, including a narrow median vitta that is bordered on either side by reddish; posterior sclerites of mesonotum dark brown, sparsely pruinose. Pleura obscure brownish yellow. Halteres elongate, dusky. Legs with the coxæ and trochanters obscure yellow; femora, tibiæ, and proximal ends of basitarsi dark brown, the remainder of tarsi white; on posterior tarsi,

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[1] Contribution from the entomological laboratory, Massachusetts State College.

the entire segment is whitened. Wings (Plate 1, fig. 1) with a strong dusky tinge, cells C and Sc, together with a seam along Cu, somewhat darker brown; stigma brown; small whitish obliterative areas before the stigma and across the basal section of M_{1+2}; veins dark brown. Venation: Rs long, exceeding R_{2+3}; forks of medial field relatively short; cell 2d A wide.

Abdominal tergites and hypopygium brownish black, the sternites brownish yellow. Male hypopygium (Plate 2, fig. 23) with the caudal margin of tergite, *9t*, transverse, with small obtuse lateral teeth and more acute submedian denticles. Outer dististyle, *od*, a broad flattened lobe, the apex subtruncate. Inner dististyle, *id*, very broad, produced into a slender apical beak, the margins heavily blackened.

MINDANAO, Davao district, Mount Apo, Baroring River, altitude 7,000 feet, November 9, 1930 (*Clagg*); holotype, male; allotype, female.

Because of the unusual length of Rs, the present species disagrees with all similar forms in the Philippine fauna. By my key to the species of this region,[2] the fly runs out at couplet 5, disagreeing with both included groups in the elongate Rs, in conjunction with the unmarked wings. In this fauna, it is closest to *haightensis* Alexander (couplet 13) in the wide cell 2d A and the distribution of white color on the legs, being well distinguished by the darkened wings, elongate Rs, and structure of the male hypopygium.

DOLICHOPEZA (NESOPEZA) INORNATIPES sp. nov. Plate 1, fig. 2; Plate 2, fig. 24.

Belongs to the *cuneata* group, *cinctitarsis* subgroup; mesonotum light brown to yellowish brown; legs black, the usual white areas much reduced in amount to almost obliterated; abdomen bicolorous; male hypopygium with the elongate tergite profoundly bifid, the mesal-apical angle of each lobe further produced into a glabrous straplike appendage.

Male.—Length, about 10 to 11 millimeters; wing, 10 to 11.

Female.—Length, about 11.5 to 12 millimeters; wing, 12 to 13.

Frontal prolongation of head yellow; basal segment of palpi pale, the remainder dark brown. Antennæ with the scape and pedicel brown, the flagellum black; flagellar segments black, with short verticils; antennæ (male) relatively long, if bent backward extending to the wing root or shortly beyond. Head light brown.

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[2] Philip. Journ. Sci. 47 (1932) 169.

Mesonotum light brown to yellowish brown, the præscutum with a narrow darker median vitta. Pleura light yellowish brown to yellow. Halteres dark brown. Legs with the coxæ, trochanters, and narrow femoral bases obscure yellow; remainder of legs black, almost unvariegated, there being only a small amount of white on extreme base of tibia, and more obscurely on the outer tarsal segments; posterior legs with the proximal ends of basitarsi restrictedly dirty white. A few specimens of the large series show an increased amount of white on the legs. Wings (Plate 1, fig. 2) with a brownish tinge, cell Sc and the oval stigma darker brown; in cases, a narrow darkened seam on anterior cord; veins dark brown. Venation: As in the *cuneata* group, cell 2d A very narrow.

Abdomen bicolorous, the segments black at the incisures, obscure yellow medially, the dark color involving both the base and apex of the individual segments. Male hypopygium (Plate 2, fig. 24) with the tergite, *9t*, long and narrow, highly arched as in the subgroup, with a deep median incision; mesal-apical angle of each lobe further produced into a slenderer, entirely glabrous, straplike portion; a narrow, elevated, blackened ridge, or carina, on ventral face near base of each tergal lobe. Ninth sternite, *9s*, broad, the caudal margin broadly and evenly emarginate.

MINDANAO, Davao district, Mount Apo (*Clagg*); holotype, male; allotype, female, altitude 7,000 feet; November 8, 1930; paratypes, 20 of both sexes, altitude 6,000 to 7,000 feet, November 8 to 10, 1930.

By my key to the Philippine species of *Dolichopeza*,[3] the present species runs to *annulitarsis* Alexander (Luzon), differing in the longer antennæ, almost uniformly blackened legs, and the more-elongate glabrous appendage on each lobe of the tergite. The amount of white on the legs is here more reduced than in any other Philippine species of the subgenus *Nesopeza*.

DOLICHOPEZA (NESOPEZA) BICORNIGERA sp. nov. Plate 2, fig. 25.

Belongs to the *cuneata* group and subgroup; male hypopygium with the lateral angles prolonged into flattened acute horns, the median region emarginate, unarmed.

Male.—Length, about 11 to 11.5 millimeters; wing, 12 to 13.

Female.—Length, 12 to 13 millimeters; wing, about 12 to 12.5.

Frontal prolongation of head brown; palpi dark brown. Antennæ (male) dark brown, the basal two segments paler; relatively elongate, if bent backward extending to some distance beyond the wing root; verticils numerous but short. Head brown, paler in front.

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[3] Loc. cit.

Mesonotum relatively dark brown; almost unvariegated. Pleura testaceous yellow. Halteres elongate, dark brown. Legs with the coxæ and trochanters testaceous yellow; femora black; tibiæ black, the extreme base whitened; tarsi white, the central portion of basitarsi extensively blackened. Wings as in the *cuneata* group; stigma dark brown; a narrow and vague brown cloud on anterior cord; veins brown. Venation as in the group.

Abdomen bicolorous, the incisures blackened, the central portion of the individual segments more yellowish; outer segments more uniformly blackened; hypopygium obscure yellow. Male hypopygium (Plate 2, fig. 25) with the tergite, *9t*, produced into conspicuous lateral flattened horns, their tips acute; median region of tergite pale, emarginate, provided with abundant yellow setæ but otherwise unarmed. Ninth sternite, *9s*, with the apex weakly emarginate, the median area membranous, the lobes densely provided with setæ, those along margin at apex short and spinose, those of the lobes very long and slender, more or less decussate across the median area.

MINDANAO, Davao district, Mount Apo, Sibulan River, altitude 2,000 feet, October 8, 1930 (*Clagg*); holotype, male; allotype, female; paratypes, 18 of both sexes.

Dolichopeza (*Nesopeza*) *bicornigera* is very different from all other described species in the peculiar structure of the male hypopygium, especially of the ninth tergite. By my key to the Philippine species of this genus,[4] the species runs out at couplet 17 by this same peculiarity of structure. This fly will presumably be found to be a low-altitude species in the mountains of the western shore of Davao Gulf.

DOLICHOPEZA (NESOPEZA) RIDIBUNDA sp. nov. Plate 2, fig. 26.

Belongs to the *cuneata* group and subgroup; mesonotum brown, without distinct markings; legs black, the tarsi conspicuously variegated with snowy white; male hypopygium with the tergite acutely toothed; ninth sternite with a deep V-shaped median notch.

Male.—Length, about 10 millimeters; wing, 11 to 11.5.

Female.—Length, about 12.5 millimeters; wing, 13.

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[4] Loc. cit.

Frontal prolongation of head brownish yellow, darker brown medially; palpi brownish black. Antennæ with the scape and pedicel light brown, the flagellum darker; antennæ (male) relatively long, if bent backward extending approximately to root of halteres; flagellar segments elongate, with short verticils; terminal segment very small. Head brown.

Mesonotum brown, without distinct markings. Pleura brownish yellow dorsally, more testaceous yellow on ventral portion. Halteres black. Legs with the coxæ and trochanters brownish yellow to yellow; femora yellow basally, passing into dark brown; tibiæ brownish black, the extreme base whitened; basitarsi mostly blackened, with about the proximal and apical fifth or sixth snowy white; remainder of tarsi white, the outer segments a trifle darkened. Wings with a faint brownish tinge, cell Sc a little darkened; stigma oval, dark brown; vague indications of a narrow brown seam on anterior cord; veins dark brown. Venation as in the group.

Abdomen ringed with black and obscure yellow, the latter color occupying the central portion of the individual segments, the dark becoming more extensive on the outer segments; hypopygium chiefly obscure yellow. Male hypopygium (Plate 2, fig. 26) with the tergite, 9*t*, relatively small, the caudal margin acutely toothed; lateral tooth slender, acute, glabrous, separated by a narrow U-shaped notch from a median plate that bears smaller acute lateral teeth and a small median denticle that is formed by the outward production of a ventral carina; surface of median plate and disk of tergite with abundant setæ. Caudal margin of ninth sternite, 9*s*, with a deep V-shaped median notch, the base of which is filled with membrane, the lobes obtusely rounded and provided with long delicate setæ.

MINDANAO, Davao district, Mount Apo, Baroring River, altitude 7,000 feet, November 9, 1930 (*Clagg*); holotype, male; allotype, female; paratype, male.

By my key to the Philippine species of *Dolichopeza*,[5] the present species runs out at couplet 18, disagreeing with both included species, *angustaxillaris* Alexander (Luzon) and *bagobo* Alexander (Mindanao), by the acute toothing of the ninth tergite and the deep median splitting of the ninth sternite. The nature of the toothing of the tergite is rather more like that of the Bornean *cuneata* Edwards, but still quite different.

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[5] Loc. cit.

LIMONIINÆ

LIMONIINI

LIMONIA (LIMONIA) MONILIS sp. nov. Plate 1, fig. 3.

Male.—Length, about 5 millimeters; wing, 6; antenna, about 2.3.

Closely allied to *Limonia* (*Limonia*) *multinodulosa* Alexander (Luzon), differing chiefly in the structure and coloration of the antennæ.

Antennæ slightly more elongate than in *multinodulosa,* the individual segments more elongate, especially the terminal segment; basal enlargement of segments black, the apical pedicels abruptly pure white, with only the extreme apex blackened; in *multinodulosa,* the base and pedicel of all segments are uniformly dark brown. Mesonotal præscutum without indications of lateral dark stripes. Wings (Plate 1, fig. 3) with Rs and R_{2+3} more elongate; distal section of M_{1+2} sinuous. Abdomen dark brown, the sternites paler. Male hypopygium very much as in *multinodulosa.*

MINDANAO, Davao district, Calian, La Lun Mountains, altitude 5,500 feet, January 1, 1931 (*Clagg*); holotype, male.

LIMONIA (LIMONIA) THETICA sp. nov. Plate 1, fig. 4; Plate 2, fig. 27.

General coloration of mesonotum reddish brown; pleura yellow, with a broad black dorsal stripe; antennæ relatively short, the flagellar segments without pedicels; legs brown, the tarsi and broad tips of all tibiæ white; wings with a brown tinge, the oval stigma darker brown; Sc_1 ending about opposite two-thirds the length of Rs; male hypopygium with the basistyle slender, the lobe basal in position.

Male.—Length, about 3.3 millimeters; wing, 3.5.

Rostrum and palpi black. Antennæ black throughout; relatively short, the flagellar segments oval, with truncated ends but no pedicels; terminal segment a little longer than the penultimate, narrowed at apex. Head dark gray.

Mesonotal præscutum reddish brown, the posterior sclerites, including the median region of scutum and the scutellum, darker brown. Pleura yellow ventrally, the dorsal portion chiefly covered by a blackish longitudinal stripe, broadest anteriorly, narrower behind, passing beneath the root of the halteres to the abdomen. Halteres with blackened knobs, the stem somewhat paler. Legs with the fore coxæ darkened, remaining coxæ and all trochanters yellow; femora yellow basally, passing to brown before midlength; tibiæ brown, the tips broadly white on all legs, the amount subequal; tarsi white; claws slender, simple or nearly so. Wings (Plate 1, fig. 4) with a brownish tinge, the oval stigma darker brown; veins dark brown. Costal fringe and macrotrichia of veins relatively long and conspicuous. Venation: Sc_1 ending about opposite two-thirds the length of Rs, Sc_2 not far from its tip; Rs long, gently arcuated; free tip of Sc_2 and R_2 in transverse alignment; m-cu shortly before fork of M; cell 2d A narrow.

Abdomen dark brown, including the hypopygium. Male hypopygium (Plate 2, fig. 27) with the tergite, *9t*, long, narrowed outwardly, the apex shallowly bilobed by a small median notch. Basistyles, *b*, elongate, the ventromesal lobe rounded, basal in position. Dorsal dististyle, *dd*, a chitinized hook, slightly expanded before the acute tip. Ventral dististyle, *vd*, a small oval lobe, set with several long coarse setæ; rostral prolongation slender, terminating in a pale spine; no rostral spines are apparent in the unique type. Gonapophyses, *g*, dusky, the mesal-apical lobe obtuse at apex.

MINDANAO, Surigao Province, Mainit, March 4, 1931 (*Duyag*); holotype, male.

Limonia (*Limonia*) *thetica* is the fourth Philippine species of the subgenus to be described in which not only the tarsi but the tips of at least the posterior tibiæ are snowy white. It differs from *L.* (*L.*) *monilis* sp. nov. and *L.* (*L.*) *multinodulosa* Alexander in the short, nonpedicellate antennæ, and in having the tips of all tibiæ whitened. Its closest ally is *L.* (*L.*) *latiflava* Alexander (Luzon), which is still known only from the female sex. The latter species has the tarsi and tibial tips more yellowish, Sc much longer, ending just before the fork of the long Rs, and cell 2d A wider.

LIMONIA (LIMONIA) SEMANTICA sp. nov. Plate 1, fig. 5; Plate 2, fig. 28.

General coloration of mesonotum reddish brown; pleura variegated with darker; antennæ (male) elongate, moniliform; all tarsi white, the proximal ends of basitarsi restrictedly darkened; halteres pale, the knobs yellow, ringed with darker at base; wings faintly tinged with brown, the oval stigma a trifle darker; male hypopygium with two rostral spines on ventral dististyle; gonapophyses entirely pale, the mesal apical angle a curved pale spine.

Male.—Length, about 3 millimeters; wing, 3.6.

Rostrum small, dark-colored; palpi pale. Antennæ (male) moniliform, the pedicels paler brown than the brownish black enlargements; basal enlargements oval to cylindrical, not at all triangular in outline, provided with long erect setæ and sparse, unilaterally arranged verticils, the latter only a trifle longer than the more-delicate setæ; apical pedicels a little shorter than the bases, becoming still shorter on the outer segments; terminal segment elongate, narrowed at apex. Head blackish.

Mesonotal præscutum reddish brown, the posterior sclerites of mesonotum somewhat darker; præscutum produced cephalad over pronotum. Pleura obscure yellow, variegated with dark brown on anepisternum and on dorsal sclerites. Halteres yellow, the base of the knob narrowly ringed with darker. Legs with the fore coxæ slightly darkened; remaining coxæ and all trochanters more yellowish; femora dark brown, the bases yellowish; tibiæ dark brown; tarsi of all legs chiefly white, the proximal ends of basitarsi infuscated, most extensively so on the fore legs, narrowest on hind legs. Wings (Plate 1, fig. 5) with a faint brown tinge, the oval stigma a trifle darker brown; veins dark. Venation: Sc_1 ending just beyond midlength of Rs, Sc_2 close to its tip; free tip of Sc_2 and R_2 both pale and in approximate transverse alignment; m-cu nearly half its length beyond the fork of M; vein Cu_2 widely separated from Cu_1.

Abdomen brown, the sternites paler. Male hypopygium (Plate 2, fig. 28) with the tergite, *9t*, transverse, the caudal margin not emarginate. Basistyle, *b*, relatively small, the mesal lobe divided into two lobules. Dorsal dististyle, *dd*, a curved chitinized rod, the apex suddenly narrowed to an acute spine. Ventral dististyle, *vd*, small, fleshy; rostral prolongation long and slender, terminating in a single elongate seta; rostral spines basal in position, two in number, placed close together. Gonapophyses, *g*, entirely pale, the mesal apical angle a gently curved pale spine.

MINDANAO, Surigao Province, Mainit, March 4, 1931 (*Duyag*); holotype, male.

Limonia (*Limonia*) *semantica* is very distinct from the other described Philippine species of the subgenus having white tarsi. The closest allies seem to be *L.* (*L.*) *candidella* Alexander and *L.* (*L.*) *subalbitarsis* Alexander, which differ conspicuously in the coloration of the body, the large size, and the structure of the antennæ and male hypopygia. The peculiar lobing of the mesal face of the basistyle of the present species is suggested by *subalbitarsis*, but the relationship between the two species does not seem to be close.

LIMONIA (LIMONIA) TRIGONELLA sp. nov. Plate 1, fig. 6.

Closely allied to *trigonia*, differing most conspicuously in the very small size.

Female.—Length, about 4.5 millimeters; wing, 5.2.

Rostrum, palpi, and antennæ black. Head black, the anterior vertex silvery.

Mesonotum dark brown, the præscutum with four more-reddish brown stripes. Pleura dark brown. Halteres black. Legs with the coxæ and trochanters ocherous yellow; femora dark brown; tibiæ and tarsi more-brownish yellow. Wings (Plate 1, fig. 6) with a brownish tinge, heavily patterned with dark brown, including five or six major costal areas, and conspicuous seams along cord and outer end of cell 1st M_2; areas in center of cell R_2 and at end of vein R_3 confluent behind, forming a Y-shaped figure, its stem crossing cells R_4, R_5, 2d M_2, and M_3; large areas at arculus and at ends of anal veins; costal areas at origin of Rs, end of Sc and at R_{1+2} very large and only narrowly separated by whitish areas; dark markings of wing conspicuously bordered by whitish.

Abdomen brownish black, the genital segments paler.

MINDANAO, Surigao Province, Mount Cantugas, March 18, 1931 (*Duyag*); holotype, female.

Limonia (*Limonia*) *trigonella* much resembles a tiny specimen of *L.* (*L.*) *claggi* Alexander or *L.* (*L.*) *trigonia* (Edwards), differing most evidently in the very small size. The male is still unknown and will very probably be found to yield distinct hypopygial characters.

LIMONIA (LIBNOTES) ASTUTA sp. nov. Plate 1, fig. 7.

General coloration of mesonotum brown, the lateral portions obscure yellow; wings with a restricted brown pattern; cells C and Sc above the origin of Rs undarkened; cell 1st M_2 shorter than any of the veins issuing from it; vein 2d A strongly arched.

Female.—Length, about 6 millimeters; wing, 7.5.

Rostrum and palpi brownish black. Antennæ brownish black throughout; flagellar segments oval, with short apical necks; verticils short, the longest unilaterally arranged. Head dark gray; anterior vertex reduced to a linear strip that is about as wide as the outer row of ommatidia.

Pronotum dark brown medially, paler laterally. Mesonotum obscure brownish yellow, the median area occupied by a broad dark brown stripe, widened behind by the confluence of the reduced lateral stripes; posterior sclerites of mesonotum dark brown, sparsely pruinose. Pleura more or less darkened, slightly pruinose, the ventral sclerites more yellowish. Halteres dark brown. Legs with the coxæ and trochanters yellow, the fore coxæ more infuscated; femora obscure yellow, passing into dark brown toward tips; tibiæ brown; tarsi broken. Wings (Plate 1, fig. 7) with a pale yellow tinge, cells C and Sc clearer yellow; a restricted dark brown pattern, arranged as follows: A darkening at arculus and in bases of cells R and M; a conspicuous oval seam at end of Sc; stigmal, over the free tip of Sc_2; cord and outer end of cell 1st M_2; R_2; a marginal seam in outer radial field; narrow brown seams on veins R_{2+3} and Cu; veins brown, pale yellow in the flavous costal areas. Macrotrichia of veins long and conspicuous. Venation: Sc_1 ending about opposite r-m, Sc_2 at its tip; free tip of Sc_2 more than its own length before R_2; veins beyond cell 1st M_2 elongate; m-cu before midlength of cell 1st M_2; vein 2d A short.

Abdominal tergites bicolorous, the bases of the individual segments brown, the apices conspicuously yellow; sternites more uniformly yellow, the subterminal segments more darkened. Ovipositor with the cerci shorter than the hypovalvæ, notched at tips; bases of hypovalvæ conspicuously blackened.

MINDANAO, Davao district, Mount Apo, Sibulan River, altitude 7,000 to 8,000 feet, September 21, 1930 (*Clagg*); holotype, female.

Limonia (*Libnotes*) *astuta* is most closely allied to *L.* (*L.*) *banahaoensis* Alexander (Luzon), differing in the body coloration, wing pattern, and venation. The restricted dark pattern of the wings does not include any portion of cells C or Sc above the origin of Rs.

LIMONIA (LIBNOTES) QUADRIPLAGIATA sp. nov. Plate 1, fig. 8; Plate 2, fig. 29.

General coloration of mesonotum dark brown, the præscutum with three more-reddish brown stripes; pleura chiefly dark brown; femora brownish yellow, passing into brown outwardly, the tip narrowly pale yellow; wings light yellow, the costal margin with four conspicuous brown areas; male hypopygium with the outer dististyle and gonapophyses heavily blackened.

Male.—Length, about 8 millimeters; wing, 9.

Rostrum and palpi brownish black. Antennæ with the basal two segments black; flagellum broken. Head gray; anterior vertex reduced to a narrow strip that is only a trifle wider than the outer row of ommatidia.

Pronotum black. Mesonotal præscutum dark brown, the three stripes more reddish brown, the median one so indicated only in front; scutal lobes conspicuously darkened; median region of scutum and the scutellum more testaceous yellow; postnotal mediotergite dark brown. Pleura chiefly dark brown, vaguely marked with restricted areas of yellowish. Halteres infuscated, the base of stem narrowly yellow; stem conspicuously fringed with long setæ. Legs with the coxæ and trochanters yellow; femora brownish yellow, darkened outwardly, the tips narrowly and abruptly yellow; tibiæ and tarsi brownish yellow, the outer tarsal segments darkened. Wings (Plate 1, fig. 8) light yellow, the costal region clearer yellow; four large and conspicuous brown costal areas, the first above the arculus, the others at origin of Rs, tip of Sc, and tip of R_{1+2}; cord and outer end of cell 1st M_2 narrowly seamed with brown; wing margin narrowly and vaguely darkened, especially in the axillary region. Venation: Sc long, Sc_1 ending about opposite m-cu, Sc_2 at its tip; basal section of R_{4+5} very short, subequal to r-m; free tip of Sc_2 and R_2 in transverse alignment; cell 1st M_2 long and narrow, m-cu at near midlength; vein 2d A beyond base gently converging toward 1st A, thence strongly diverging to margin.

Abdomen bicolorous, dark brown, the caudal margins of the individual segments conspicuously yellow; hypopygium more yellowish. Male hypopygium (Plate 2, fig. 29) with the tergite, *9t*, deeply emarginate medially, each lobe obtusely rounded. Dorsal dististyle, *dd*, a powerful, slightly curved, blackened rod. Ventral dististyle, *vd*, a small suboval lobe, its mesal face produced into two lobules, as figured. Gonapophyses, *g*, with the mesal apical region produced into a powerful blackened beak.

MINDANAO, Davao district, Mount Apo, Baroring River, altitude 6,000 feet, November 10, 1930 (*Clagg*); holotype, male.

By existing keys to the subgenus *Libnotes*, the present species runs to *L.* (*L.*) *longinervis* (Brunetti), from the Indian Himalayas. It is distinguished by the darker coloration of the body, and the different wing coloration and pattern, together with details of venation, as the much longer vein Sc and cell 1st M_2.

LIMONIA (LIBNOTES) HENRICI sp. nov. Plate 1, fig. 9.

Head and thorax, with all appendages, black; wings strongly suffused with brown, the costal region more blackened; abdomen red, the basal tergite and genital segments of both sexes black.

Male.—Length, about 9 millimeters; wing, 10.

Female.—Length, about 12 millimeters; wing, 12.

Head, including appendages, black.

Entire thorax, including halteres and legs, black. Wings (Plate 1, fig. 9) with a very strong brown tinge, the prearcular region, cells C and Sc, and the stigma blackish; veins brown to dark brown. Venation: Sc long, Sc_1 ending nearly opposite m-cu, Sc_2 at its tip; Rs oblique, nearly straight to weakly sinuous; free tip of Sc_2 far before R_2, the distance longer than m-cu; m-cu at near midlength of cell 1st M_2; vein 2d A long, at base converging toward 1st A.

Abdomen with the basal tergite and genital segments of both sexes black, the intermediate region bright red, the lateral line narrowly blackened in male; hypopygium black; valves of ovipositor horn-colored.

MINDANAO, Davao district, Mount Apo, Galog River, altitude 6,000 feet (*Clagg*); holotype, male, October 27, 1930; allotype, female, November 6, 1930.

Limonia (*Libnotes*) *henrici* is named in honor of Mr. J. Henry Clagg, father of the collector of the types. It belongs to the *semperi* group of the subgenus, differing notably from the other included species in having the head and thorax intense black, instead of orange.

LIMONIA (ALEXANDRIARIA) NATHALINÆ sp. nov. Plate 1, fig. 10; Plate 2, fig. 30.

General coloration of mesonotum polished black, including the præscutal shield; pleura yellow, the anepisternum and pteropleurite black, the pleurotergite gray; wings tinged with brown, the prearcular region more yellowish.

Male.—Length, about 4.5 millimeters; wing, 5.

Rostrum and palpi black. Antennæ black throughout; flagellar segments short-oval, the terminal segment not elongated. Head black, the broad anterior vertex and narrow posterior orbits silvery.

Pronotum black, paler laterally. Mesonotal præscutum almost covered by a polished black shield, only the humeral triangles obscure yellow; scutal lobes polished black; median region of scutum and the scutellum testaceous-yellow; postnotal mediotergite black, more or less pruinose. Pleura obscure yellow, the anepisternum and pteropleurite polished black, the pleurotergite heavily gray pruinose. Halteres with the stem pale, the knobs dark brown. Legs with the coxæ and trochanters yellow; femora yellow, the tips narrowly infuscated; tibiæ and tarsi brownish black. Wings (Plate 1, fig. 10) tinged with brownish, the prearcular region more yellowish; stigma very pale brown. Venation: Sc_1 ending far before origin of Rs, the distance about one-half longer than Rs; Sc_1 long; Rs short, a little more than one-half the basal section of R_{4+5}.

Abdomen dark brown, the ventral dististyle of the hypopygium paler. Male hypopygium (Plate 2, fig. 30) with the caudal margin of the tergite, *9t*, very deeply notched, the lateral angles produced into slender lobes that are tipped with long black setæ. Ventral dististyle, *vd*, larger than in *argyrata*, the rostral spines somewhat longer, exceeding the prolongation in length.

MINDANAO, Davao district, Calian, La Lun Mountains, altitude 5,500 feet, December 29, 1930 (*Clagg*); holotype, male.

Limonia (*Alexandriaria*) *nathalinæ* is named in honor of Mrs. Nathalin Clagg, mother of the collector, Mr. Charles F. Clagg, whose various trips to Mount Apo have added greatly to our knowledge of the distribution of Tipulidæ in Mindanao. It is most closely allied to *L.* (*A.*) *argyrata* Alexander (Formosa, Luzon) differing in the blackened mesonotum, the extensive black areas on the thoracic pleura, and details of structure of the male hypopygium, as the narrower lateral lobes of the tergite and the longer spines of the rostral prolongation of the ventral dististyle.

LIMONIA (ALEXANDRIARIA) TECTA sp. nov. Plate 1, fig. 11; Plate 3, fig. 31.

General coloration yellow, the mesonotal præscutum with a single median black stripe that widens out at the suture, being evidently formed by the confluence of the short laterals with the usual median area; femora obscure yellow, the tips narrowly dark brown; wings subhyaline, the stigma dark brown, clearly defined; Sc_1 very long, exceeding the distal section of M_{1+2}; Rs shorter than the basal section of R_{4+5}.

Male.—Length, about 3.5 millimeters; wing, 4.5.

Female.—Length, about 3.5 millimeters; wing, 4.2.

Rostrum obscure yellow; palpi brownish black. Antennæ brownish black throughout; flagellar segments oval, the terminal segment elongate. Head light brown.

Mesonotal præscutum obscure yellow, the median area with a single black stripe that widens at the suture; a small median area of yellow behind this stripe on præscutum; scutal lobes brownish black, the median region yellow; scutellum and postnotal mediotergite chiefly darkened. Pleura light yellow. Halteres yellow, the knobs dark brown. Legs with the coxæ and trochanters yellow; femora obscure yellow, the tips narrowly dark brown; tibiæ brown, the tarsi paling to yellow. Wings (Plate 1, fig. 11) subhyaline, the stigma dark brown, clearly defined; veins brownish black. Venation: Sc_1 ending some distance before origin of Rs, the distance on costa being longer than Rs alone; Sc_1 very long, exceeding the distal section of M_{1+2}; Rs shorter than the basal section of R_{4+5}; cell 2d A relatively narrow, the outer end pointed.

Abdominal tergites dark brown, the sternites paler, the more proximal sternites with their bases darkened. Male hypopygium (Plate 3, fig. 31) with the tergite, *9t*, transverse, the caudal margin very gently emarginate. Ventral dististyle, *vd*, a large fleshy lobe, the rostral prolongation bearing a stout powerful spine that is evidently formed by the fusion or very close approximation of two spines.

MINDANAO, Davao district, Mount Apo, Galog River, altitude 6,000 feet (*Clagg*); holotype, female, September 26, 1930; allotype, male, September 9, 1930.

Limonia (*Alexandriaria*) *tecta* differs from the somewhat similar *L.* (*A.*) *argyrata* Alexander (Formosa, Luzon) and *L.* (*A.*) *nathalinæ* sp. nov. in the pattern of the head and thorax, the dark brown, clearly defined stigma, and, especially, the structure of the male hypopygium, notably the feebly emarginate tergite. *Limonia* (*A.*) *brevissima* Alexander (Luzon) has a similarly darkened stigmal area, but is very different in the unusually short, transverse Rs. The present species has Sc_1 longer than any other regional species of the subgenus.

HELIUS (HELIUS) COSTOSETOSUS sp. nov. Plate 1, fig. 12; Plate 3, fig. 32.

General coloration dark brown; legs black, the tarsi paling to brownish yellow; wings grayish subhyaline, cells C and Sc more yellowish; a conspicuous erect costal fringe; cell 1st M_2 small, with m-cu at near midlength.

Male.—Length, about 5.5 to 5.7 millimeters; wing, 6 to 6.2.

Female.—Length, about 6 to 6.5 millimeters; wing, 5.5 to 5.8.

Rostrum dark brown, a little longer than the remainder of head; palpi brownish black. Antennæ dark brown throughout; flagellar segments oval, clothed with a dense erect pubescence and short verticils. Head brownish gray.

Mesonotum chiefly dark brown, the humeral region somewhat brighter. Pleura chiefly dark brown, the ventral sternopleurite a little brightened. Halteres brownish black, the base of the stem narrowly pale. Legs with the coxæ brownish yellow; trochanters obscure yellow; femora yellow at base, the remainder passing to black; tibiæ black; tarsi chiefly brownish yellow, the outer segments brightening to yellow. Wings (Plate 1, fig. 12) grayish subhyaline, cells C and Sc more yellowish; stigma elongate, pale brown; veins dark brown. Costal margin of both sexes, but more especially of the male, with a long erect fringe of setæ. Venation: Sc_1 ending nearly opposite the fork of Rs, Sc_2 at its tip; anterior branch of Rs sinuous, on distal third running close to vein R_{1+2}, the two elements relatively close together at margin; m subequal to the second section of M_{1+2} or the basal section of M_3; m-cu near or beyond midlength of cell 1st M_2.

Abdominal tergites dark brown, the basal sternites yellowish, the outer segments more darkened; hypopygium light brown. Male hypopygium (Plate 3, fig. 32) with the basistyle, *b*, relatively slender. Outer dististyle, *od*, a nearly straight rod, the apex more blackened and weakly bidentate. Inner dististyle, *id*, with the basal two-thirds more enlarged, provided with coarse erect setæ, the distal third narrowed to a flattened bladelike portion that is provided with small delicate punctures. Ædeagus, *a*, long, forming a weak coil.

MINDANAO, Surigao Province, Mount Diuata, April 10 to 17, 1931 (*Duyag*); holotype, male; allotype, female; numerous paratypes of both sexes.

By my key to the Philippine species of *Helius*,[6] the present species runs to couplet 10, where it disagrees with all species in the venation, notably the shape of cell 1st M_2 and the position of m-cu at near midlength of the cell. The long conspicuous costal fringe likewise distinguishes the fly from other forms found in the Islands. From the somewhat similar *H.* (*H.*) *costofimbriatus* Alexander (Riukiu Islands), the present fly is readily told by the details of venation, as the position of m-cu.

HELIUS (HELIUS) DEVINCTUS sp. nov. Plate 1, fig. 13; Plate 3, fig. 33.

General coloration dark brown; antennæ (male) elongate, if bent backward extending nearly to base of abdomen; legs black, the tarsi paling to brownish yellow; wings with a faint brown tinge, cells C and Sc, with the stigma, darker brown; costal fringe short and inconspicuous; m-cu at near midlength of cell 1st M_2; male hypopygium with a hairy lobe on mesal face of basistyle at or beyond midlength.

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[6] Philip. Journ. Sci. 47 (1932) 184.

Male.—Length, about 4 to 4.5 millimeters; wing, 4.5 to 5.

Rostrum brownish black, somewhat longer than the remainder of head; palpi black. Antennæ brownish black, in male of a somewhat unusual length for a member of this genus of flies, if bent backward extending nearly to base of abdomen; flagellar segments cylindrical, clothed with a dense erect pubescence. Head black.

Mesonotum chiefly dark brown, the præscutum vaguely lined with paler, especially near suture; scutellum obscure yellow, darkened medially at base. Pleura conspicuously dark brown on dorsal portion, the ventral sclerites and pteropleurite a little paler. Halteres brownish black. Legs with the coxæ and trochanters yellow to brownish yellow, the fore coxæ somewhat darker; remainder of legs black, the outer tarsal segments paling to brownish yellow. Wings (Plate 1, fig. 13) with a faint brown tinge, cells C and Sc, together with the stigma, darker brown; veins dark brown. Costal fringe short and inconspicuous. Venation: Anterior branch of Rs strongly arcuated at origin, on distal two-thirds extending generally parallel to R_{1+2}; m-cu at or beyond midlength of cell 1st M_2, in approximate transverse alignment with r-m.

Abdominal tergites dark brown, the sternites more reddish brown, the caudal margins of the intermediate segments a trifle darkened; hypopygium yellowish brown. Male hypopygium (Plate 3, fig. 33) with the basistyle, *b*, relatively slender, the mesal face on distal half with a conspicuous hairy lobe. Outer dististyle, *od*, a very slender, nearly straight rod, the apex entire or nearly so. Gonapophyses, *g*, appearing as very slender curved spines from a dilated base.

MINDANAO, Surigao Province, Mainit, March 21 to 27, 1931 (*Duyag*); holotype, male; allotype, female; paratypes, several of both sexes; Mount Cantugas, March 19, 1931 (*Duyag*); paratypes, several of both sexes.

Helius (*Helius*) *devinctus* is generally similar to *H.* (*H.*) *costosetosus* sp. nov., differing most evidently in the elongate antennæ of the male, short costal fringe in both sexes, and the very different structure of the male hypopygium. By my key to the Philippine species of *Helius*,[7] this fly runs to exactly the same place as does *costosetosus,* differing from the various included species in the same characters. The presence of the hairy lobe on the basistyle of the male hypopygium suggests the condition found in the Formosan *H.* (*H.*) *tenuistylus* Alexander and allied species, but the present fly is very different in venation and in details of structure of the hypopygium.

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[7] Loc. cit.

HEXATOMINI

ULA AURITARSIS sp. nov. Plate 1, fig. 14.

General coloration dark brown, including the knobs of halteres; legs brown, the tarsi and narrow tips of the middle and hind tibiæ light yellow; wings strongly infuscated; cell 1st M_2 very small.

Male.—Length, about 6 millimeters; wing, 7.

Female.—Length, about 7 millimeters; wing, 7.

Rostrum and palpi black. Antennæ with the scape and pedicel dark brown, the flagellum black; flagellar segments long-oval. Head dark gray.

Pronotum brownish gray. Mesonotum dark brown, the humeral region of præscutum obscure yellow; median region of scutum and the scutellum obscure brownish yellow, the latter slightly infuscated along caudal margin. Pleura chiefly dark brown, the pteropleurite, ventral sternopleurite, and meral region more yellowish. Halteres obscure yellow, the knobs dark brown. Legs with the coxæ and trochanters obscure yellow; femora brownish yellow, the tips passing to darker brown; tibiæ pale brown, the tips of middle and hind tibiæ narrowly light yellow; tarsi light yellow, the proximal ends of fore basitarsi infuscated. Wings (Plate 1, fig. 14) with a strong brown tinge, the stigma darker; wing base slightly yellowish; veins dark brown. Macrotrichia of cells very abundant beyond cord, in cell M continued basad as a central line of trichia, and along caudal margin of wing as a marginal series in both anal cells. Venation: Cell 1st M_2 very small.

Abdominal tergites dark brown, the sternites more yellowish, subterminal segments ringed with dark brown; hypopygium obscure yellow.

MINDANAO, Davao district, Calian, La Lun Mountains, altitude 5,500 feet, at trap lantern, January 1, 1931 (*Clagg*); holotype, male; allotype, female.

Ula auritarsis is readily told from *U. mindanica* by the characters given in the accompanying key to the Philippine species of *Ula.*

Key to the Philippine species of Ula.

1. Legs, including the tarsi, dark brown................ *U. mindanica* Alexander.
Legs dark brown, the tarsi abruptly light yellow.
U. auritarsis sp. nov.

LIMNOPHILA (LIMNOPHILA) PETULANS sp. nov. Plate 1, fig. 15; Plate 3, fig. 34.

Antennæ with the scape and pedicel brownish black, the flagellum yellow; general coloration of thorax brownish black; knobs of halteres dark brown; legs yellow, the femora with a very vague darker subterminal ring; wings with the ground color pale yellow, with a heavy brown, much broken, ocellate pattern; abdomen, including the hypopygium, dark brown.

Male.—Length, about 5 millimeters; wing, 5.8.

Rostrum and palpi dark brown. Antennæ short; scape and pedicel brownish black, the flagellum pale yellow, the outer segments a trifle darker. Head brownish gray; anterior vertex broad.

Mesonotal præscutum brown, more blackish laterally; posterior sclerites of mesonotum brownish black, sparsely pruinose. Pleura brownish black. Halteres dusky, the knobs dark brown. Legs with the coxæ and trochanters dark brown; femora yellow, with a narrow, vaguely darker ring before the tip; tibiæ and tarsi yellow; the segments with long conspicuous yellow setæ. Wings (Plate 1, fig. 15) with the ground color pale yellowish, very heavily patterned with brown, this pattern arranged chiefly as much broken ocelli, centering about the origin of Rs, the cord, outer end of cell 1st M$_2$, and the center of the radial field; in addition, all cells of wing with additional spots and dots of brown, those in the costal cell appearing as narrow transverse lines. Costal fringe relatively short. Wing widest just beyond the termination of vein 2d A. Venation: Sc$_1$ ending opposite fork of Rs, Sc$_2$ at its tip; R$_2$ subequal to R$_{1+2}$; veins R$_3$ and R$_4$ diverging strongly at margin, cell R$_3$ correspondingly widened; m-cu at near one-third the length of cell 1st M$_2$; vein 2d A ending just basad of the widest part of wing.

Abdominal tergites dark brown, sparsely yellow pollinose; sternites with the central portion obscure yellow, bordered on all sides with darker; hypopygium brownish black. Male hypopygium (Plate 3, fig. 34) with the outer dististyle, *od*, a simple rod, curved at apex to an acute point, the outer fourth or fifth with microscopic setulæ. Inner dististyle, *id*, broad. Gonapophyses, *g*, appearing as long, paddlelike blades.

MINDANAO, Davao district, Mount Apo, Poraka River, altitude 6,500 feet, September 8, 1930 (*Clagg*); holotype, male.

Limnophila (Limnophila) petulans is most nearly allied to *L. (L.) murudensis* Edwards (Borneo), differing especially in the uniformly darker body coloration, the dark brown knobs of the halteres, the uniformly yellow tibiæ and tarsi, more-broken ocellate wing pattern, and blackened hypopygium.

Genus PILARIA Sintenis

Subgenus EUPILARIA novum

Generally similar to *Pilaria*, s. s., differing especially in the venation of the radial field of the wing. Tuberculate pits and pseudosutural foveæ present but pale. Antennæ with basal flagellar segments short and crowded, the outer segments with long conspicuous verticils. Wings with the anterior branch of Rs short, about one-third the posterior branch, cell R_3 at margin being some five times as wide as cell R_2; cell M_1 lacking.

Type of the subgenus, *Pilaria phœnosoma* Alexander (Oriental Region).

Besides the type, *Pilaria auranticolor* sp. nov. and *P. leucopeza* Alexander belong to this group. The three known species are found only in the Philippines, but the group will very probably be found to have a much wider range in the Oriental Region.

PILARIA (EUPILARIA) AURANTICOLOR sp. nov. Plate 1, fig. 16; Plate 3, fig. 35.

General coloration fiery orange; antennæ black, the scape brownish yellow; all tarsi brown; wings strongly tinged with fulvous; abdomen orange; male hypopygium with the outer dististyle slender, weakly bidentate at apex; each gonapophysis terminating in two conspicuous spines.

Male.—Length, about 8 millimeters; wing, 8.2.

Head deep orange. Antennæ with the scape brownish yellow, the remainder, including the pedicel, black.

Thorax fiery orange, the scutal lobes and scutellum weakly pruinose. Halteres yellow, the knobs dark brown. Legs with the coxæ and trochanters yellow; femora obscure yellow, the tips narrowly infuscated; tibiæ and tarsi brown. Wings (Plate 1, fig. 16) with a strong fulvous tinge, much brighter than in *phœnosoma;* prearcular and costal regions more yellowish; stigma small, a little darker than the ground color.

Abdomen fiery orange, including the hypopygium. Male hypopygium (Plate 3, fig. 35) with the tergite, *9t*, only slightly produced medially, the central portion irregularly notched, as shown, the lobules formed being densely set with microscopic setulæ. Outer dististyle, *od*, slender, gently curved near apex, the tip weakly bidentate. Gonapophyses, *g*, slender, each terminating in two spines.

MINDANAO, Surigao Province, Mainit, March 4, 1931 (*Duyag*); holotype, male.

Pilaria (*Eupilaria*) *auranticolor* is closely related to *P.* (*E.*) *phœnosoma* Alexander (Luzon), differing most evidently and decisively in the structure of the male hypopygium, especially the conformation of the tergal margin, the slender outer dististyle, and the conspicuous spinous gonapophyses.

ERIOPTERINI

GONOMYIA (LIPOPHLEPS) SAGITTIFERA sp. nov. Plate 1, fig. 17; Plate 3, fig. 36.

Belongs to the *skusei* group; general coloration dark brown; antennæ brownish black throughout; pleura striped longitudinally with whitish; knobs of halteres obscure yellow; legs brownish black; wings brownish gray, the stigma ill-defined; Sc long, Sc_1 ending about opposite one-third the length of Rs; male hypopygium with the outer dististyle a very long, slender, blackened hook.

Male.—Length, about 3.4 to 3.5 millimeters; wing, 4.2 to 4.3.

Rostrum brown; palpi brownish black. Antennæ brownish black throughout, relatively elongate for a member of this group; flagellar segments long-cylindrical, clothed with numerous erect setæ. Head brownish black.

Pronotum pale yellowish white. Mesonotal præscutum dark brown, sparsely pruinose with gray, the extreme lateral margin yellow; pseudosutural foveæ polished, dark colored; median region of scutum obscure yellow, darker behind; scutellum light yellow; postnotal mediotergite brownish gray, the extreme anterolateral portions yellow. Pleura dark brown, sparsely pruinose, with a broad longitudinal whitish stripe occupying the ventral pleurites; sclerites surrounding the wing root obscure yellow. Halteres dusky, the knobs yellow. Legs with the coxæ obscure brownish yellow; trochanters obscure yellow; remainder of legs brownish black. Wings (Plate 1, fig. 17) with a faint brownish gray suffusion, the stigmal region weakly darker; veins dark brown. Venation: Sc long, Sc_1 ending about opposite one-third the length of Rs, Sc_2 some distance from its tip; cell 1st M_2 closed; m-cu near fork of M.

Abdominal tergites dark brown, the sternites obscure yellow; hypopygium obscure yellow. Male hypopygium (Plate 3, fig. 36) with the basistyle, *b*, terminating in two fleshy lobes. Outer dististyle, *od*, a very long, slender, blackened hook that is curved to the acute point. Inner dististyle a straight to slightly arcuated pale club, the apex of the style slightly dilated and bearing a stout fasciculate seta at inner apical angle. Phallosome, *p*, a massive flattened structure, the ædeagus elongate.

MINDANAO, Davao district, Mount Apo, Sibulan River, altitude 7,000 to 8,000 feet, September 21, 1930 (*Clagg*); holotype, male; paratypes, two males.

Gonomyia (*Lipophleps*) *sagittifera* is very distinct from *G.* (*L.*) *acanthophallus* Alexander (Mindanao) and *G.* (*L.*) *longiradialis* Alexander (Luzon), the most nearly allied regional species of the group, in the large, powerfully constructed male hypopygium.

GYMNASTES (PARAGYMNASTES) HYLÆA sp. nov. Plate 1, fig. 18; Plate 3, fig. 37.

General coloration of mesonotal præscutum brownish yellow, with three nearly confluent shiny black stripes; head yellow; femora black, with a narrow yellow subterminal ring; wings whitish subhyaline, crossbanded with dark brown, including a narrow band at level of origin of Rs, one at cord and the broad apex; cell R_2 very small, strongly narrowed to closed at outer end.

Male.—Length, about 5.5 millimeters; wing, 5.2.

Rostrum and palpi black. Antennæ with the scape and pedicel obscure yellow; flagellum black; flagellar segments short-oval, with elongate, unilaterally arranged verticils; outer segments more elongate. Head obscure yellow.

Pronotum obscure yellow. Mesonotal præscutum brownish yellow, largely covered by three nearly confluent shiny black stripes; lateral margin of præscutum behind the pseudosutural foveæ more obscurely darkened; posterior sclerites of mesonotum chiefly blackened. Pleura dull black, the ventral sternopleurite more reddish brown. Halteres blackened, the apices of the knobs obscure yellow. Legs with the coxæ blackened; trochanters brownish yellow; femora obscure yellow at base, blackened outwardly, with a narrow yellow subterminal ring at nearly three times its width from apex; tibiæ and tarsi blackened. Wings (Plate 1, fig. 18) whitish subhyaline, heavily patterned with dark brown, arranged chiefly as three transverse bands, the basal one at level of origin of Rs narrow and nearly parallel-sided; second band at cord, widest near costa; apical area broadest; cells C and Sc darkened; an isolated brown area covering outer end of cell 1st M_2; vein Cu and axilla weakly seamed with brown. Costal fringe conspicuous. Venation:

Cell R_2 reduced to a tiny triangle by the approximation of veins R_{1+2} and R_3.

Abdominal tergites black, the caudal margins of the segments restrictedly pale; sternites similar, the yellow incisures somewhat more extensive in area. Male hypopygium (Plate 3, fig. 37) with the dististyle, *d*, a simple, obtusely rounded plate. Ædeagus, *a*, an elongate compressed blade.

MINDANAO, Surigao Province, Mainit, March 4, 1931 (*Duyag*); holotype, male.

Gymnastes (*Paragymnastes*) *hylæa* is most nearly allied to *G.* (*P.*) *pictipennis* Edwards (Siam) in the general pattern of the legs and wings, and the venation, especially the much-reduced cell R_2. It differs in the variegated coloration of the thorax and abdomen, the wing pattern, especially the narrow basal fascia, and the structure of the male hypopygium, especially of the dististyle.

I have here restricted the subgeneric term *Gymnastes* to include only the species having cell R_2 entirely obliterated by the fusion of veins R_2, R_3, and R_{1+2}. The more-numerous species of the genus having cell R_2 present are placed in the modified subgenus *Paragymnastes* Alexander, which has hitherto included only species from the Australasian Region. The distinctions between the two groups are admittedly very weak but parallel corresponding distinctions found in the closely allied genera *Teucholabis* Osten Sacken (with *Paratropesa* Schiner) and *Gonomyia* Meigen (with *Lipophleps* Bergroth and *Ptilostenodes* Alexander). *Gymnastes* seems distinct from *Teucholabis* in the scaly covering of the legs and in the manner in which cell R_2 has been lost by the approximation and fusion back from the margin of the veins that inclose it.

TEUCHOLABIS (TEUCHOLABIS) RUTILANS sp. nov. Plate 1, fig. 19; Plate 3, fig. 38.

General coloration of thorax polished reddish orange; head black, sparsely pruinose; antennæ black throughout; halteres black; legs black, the femora with a conspicuous yellow ring on apical half; wings with slightly more than the basal half whitish, the distal portion and costal region dark brown; Sc long; cell 1st M_2 closed; abdomen black.

Male.—Length, about 7.5 millimeters; wing, 7.5.

Rostrum and palpi black. Antennæ black throughout; basal flagellar segments globular, the smaller outer segments passing into oval; verticils of moderate length, exceeding the segments. Head black, sparsely pruinose, especially on anterior vertex.

Pronotum, mesonotum, and pleura polished reddish orange, the meral region blackened. Halteres short, brownish black. Legs with the fore and middle coxæ reddish orange, the posterior coxæ and trochanters black; femora black, with a conspicuous yellow ring beyond midlength; tibiæ and tarsi black; legs stout and conspicuously hairy. Wings (Plate 1, fig. 19) with the costal region and approximate distal half of wing dark brown, the proximal portions whitish; the darkened areas include the prearcular region, cells C and Sc, the radial field except the proximal ends of cells R$_1$, R$_4$, and R$_5$, and a linear streak in center of cell R$_2$; distal third of cell 1st M$_2$ and outer three-fourths of cell M$_4$ darkened; veins dark. Venation: Sc long, Sc$_1$ ending at near two-thirds the length of Rs, Sc$_2$ at near midlength of origin of Rs and tip of Sc$_1$; veins R$_4$ and R$_5$ long and extending generally parallel to one another; cell 1st M$_2$ closed; m-cu less than its length beyond fork of M.

Abdomen, including hypopygium, black. Male hypopygium (Plate 3, fig. 38) with the outer apical angle of basistyle, *b*, produced into a slender black spine. Dististyle, *d*, as shown, the outer apical angle produced into a slender lobe that bears two setæ.

MINDANAO, Davao district, Mount Apo, Sibulan River, altitude 2,000 feet, October 11, 1930 (*Clagg*); holotype, male.

Teucholabis (*Teucholabis*) *rutilans* is very different from the remaining species of the genus in the Philippines. It is somewhat similar in general features to *T.* (*T.*) *plecioides* de Meijere (Java), differing especially in the pattern of the legs and wings. The known Philippine species of the genus may be separated by the following key:

Key to the Philippine species of Teucholabis.

1. Cell 1st M$_2$ open by atrophy of basal section of M$_3$ .... 2.
   Cell 1st M$_2$ closed .... 3.
2. Cell 2d M$_2$ relatively shallow, shorter than its petiole. (Luzon.)
   T. (*T.*) *confluenta* Alexander.
   Cell 2d M$_2$ deep, about one-half longer than its petiole. (Mindanao.)
   T. (*T.*) *confluentoides* Alexander.
3. Wings pale yellow, the narrow apex and a crossband at cord pale brown; costal fringe (male) long and conspicuous. (Luzon.)
   T. (*T.*) *quinquemaculata* Alexander.
   Wings either uniformly darkened (*majuscula*) or else dimidiate, the basal half whitish, the distal portion darkened (*rutilans*); costal fringe short in both sexes .... 4.
4. Legs uniformly blackened; wings uniformly suffused with yellowish brown. (Mindanao.) .... *T.* (*T.*) *majuscula* Alexander.

Legs black, the femora with a conspicuous yellow ring on distal half; wings dimidiate, whitish, the distal half or a little less darkened. (Mindanao.) .... *T.* (*T.*) *rutilans* sp. nov.

TAIWANINA MINDANICA sp. nov. Plate 1, fig. 20.

General coloration dark brown; pronotum obscure yellow; a silvery white longitudinal stripe on thoracic pleura; halteres yellow, the knobs blackened; femora black, the bases yellowish, most extensively so on posterior legs; wings cream-yellow, with a handsome brown pattern, arranged more or less reticulately; distal section of Cu_1 short, about one-half m-cu; abdominal sternites two to six, inclusive, with paired whitish spots at base.

Male.—Length, about 6.5 millimeters; wing, 6.4.

Rostrum nearly as long as remainder of head, black, the mouth parts much reduced to virtually lacking. Antennæ with the scape and pedicel black, the flagellum brownish yellow, passing into darker brown outwardly; fusion segment elongate, involving about seven or eight segments, the outer flagellar segments with their sutures lacking or nearly so, delimited chiefly by constrictions and the arrangement of the long conspicuous verticils; terminal segment slender, linear. Eyes very large, with coarse ommatidia; vertex reduced to a narrow strip; eyes broadly contiguous beneath. Head dark brownish gray.

Pronotum obscure yellow, with a brown lateral spot. Mesonotal præscutum strongly produced over the pronotum, as in the genus, dark brown, narrowly bordered in front with yellow, the brown passing into brownish black laterally, forming an abrupt contrast with the yellow border; posterior sclerites of notum dark brown. Pleura black, with a broad silvery white longitudinal stripe extending from the propleura, passing beneath the root of the halteres; dorsopleural membrane pale yellow, variegated with brownish black. Halteres pale yellow, the knobs blackened. Legs with the coxæ and trochanters brownish black; bases of femora narrowly yellow, passing into black, the amount of yellow most extensive on the posterior legs where more than the basal half is included; tibiæ yellow, the base and apex narrowly whitened; basitarsi yellow, narrowly dark brown at proximal end; remainder of tarsi yellow. Wings (Plate 1, fig. 20) with the ground color cream-yellow, handsomely patterned with dark brown and grayish brown, the darkened areas more or less reticulate; large areas at origin of Rs and stigma, with narrow seams along cord and outer end of cell 1st M_2, connected with a reticulate area in center of outer radial field;

bases of cells R and M restrictedly darkened; a dusky wash in bases of cells M and Cu; dark marginal clouds at near midwidth of anal cells; veins black, C, Sc, and R light yellow. Venation: Much as in *pandoxa,* differing in certain details; Rs spurred at origin; distal section of Cu_1 very short, about one-half m-cu and diverging strongly from it; anal veins bent strongly to margin.

Abdomen black; sternites two to six, inclusive, with conspicuous paired whitish spots at base; hypopygium black.

MINDANAO, Davao district, Mount Apo, Baroring River, altitude 7,000 feet, November 8, 1930 (*Clagg*); holotype, male.

The discovery of a second species of *Taiwanina* is of much interest. The type and only previously known species is *T. pandoxa* Alexander (Formosa). In characters the present species conforms very closely to the genotype and so adds little to our knowledge of the exact position of the group. The fusion segment of the antennæ is even more profound than in *pandoxa,* and the sutures between the remaining flagellar segments are scarcely indicated. The short rostrum, with greatly reduced mouth parts, the anteriorly produced præscutum, and the venation are all much as in the genotype. In the structure of the head, as the rostrum and antennæ, and of the thorax, as the produced præscutum and the extensive sternopleurite, the resemblance to *Toxorhina* is well indicated. I cannot but feel that the group is closer to *Toxorhina* than to the Heliaria, despite the simple setæ of the legs and the general structure of the male hypopygium, the latter being similar in general features to members of the Heliaria. *Taiwanina mindanica* is readily told from *T. pandoxa* by the diversified wing pattern and the very short distal section of vein Cu_1.

TRENTEPOHLIA (MONGOMA) PERSIMILIS sp. nov.

Male.—Length, 8 to 9 millimeters; wing, 7.5 to 8.

Female.—Length, 8.5 to 9 millimeters; wing, 8.

Very similar to *T.* (*M.*) *brevifusa* Alexander (Luzon), differing chiefly in the longer legs and darker coloration of the body. Mesonotum uniformly dark brown, the ventral pleurites paler. Setæ of body longer and more conspicuous, including the two groups on the sternopleurite and the series on the præscutal interspaces. Apical fusion of veins Cu_1 and 1st A very slight to almost lacking.

MINDANAO, Surigao Province, Mainit (*Duyag*); holotype, male, March 22, 1931; allotype, female, March 25, 1931; paratopotype, 1 male, with allotype; paratypes, males and females, Mount Cantugas, March 17, 1931; males and females, Diuata Mountains, April 10, 1931.

TRENTEPOHLIA (MONGOMA) ALBOTERMINALIS sp. nov. Plate 1, fig. 21.

General coloration of mesonotum dark brown; head black, with median carina on vertex; legs dark brown, the tarsi and tips of all tibiæ white; wings grayish subhyaline; inner end of cell M_3 lying far proximad of cells R_5 or 2d M_2.

Male.—Length, 5.5 to 6 millimeters; wing, 5 to 6.

Rostrum yellowish brown. Antennæ brownish black throughout. Head black, the vertex with a median carina.

Mesonotum dark brown, the præscutum paler laterally. Pleura obscure yellow, the dorsal portions darker. Halteres short, dusky, the extreme base of stem yellow. Legs with the coxæ and trochanters obscure yellow; femora and tibiæ dark brown, the tips of the latter abruptly white, the amount subequal on all legs and including about the outer tenth of the segment; tarsi white; fore femora on basal portion with a few erect stout setæ. Wings (Plate 1, fig. 21) grayish subhyaline, the costal region narrowly darker; veins brown. Venation: Cell R_3 large; inner end of cell M_3 lying far proximad of either R_5 or 2d M_2; apical fusion of veins Cu_1 and 1st A slight.

Abdomen, including hypopygium, dark brown.

MINDANAO, Surigao Province, Mainit (*Diiyag*); holotype, male, March 24, 1931; paratype, male, March 23, 1931.

Trentepohlia (*Mongoma*) *alboterminalis* is readily told from the other described regional species of the subgenus *Mongoma* by the conspicuous whitened tips of all tibiæ. By my latest key to the Philippine species of *Trentepohlia*,[8] the present species runs to *T.* (*M.*) *pennipes* (Osten Sacken), a very different species with feathered mid-tibiæ.

TRENTEPOHLIA (ANCHIMONGOMA) BEATA sp. nov. Plate 1, fig. 22.

General coloration brownish black; legs brownish black, the tarsi chiefly snowy white; wings with more than the cephalic half strongly suffused with blackish, the posterior portions more grayish.

Male.—Length, about 6 millimeters; wing, 5 to 5.5.

Female.—Length, 6 to 6.5 millimeters; wing, 5 to 5.5.

Rostrum and palpi black. Antennæ black throughout, relatively elongate in male, if bent backward extending about to

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[8] Philip. Journ. Sci. 43 (1930) 297–298.

base of abdomen; flagellar segments cylindrical, with verticils [illegible] than the segments. Head black.

Mesonotum almost uniformly dark brown. Pleura dark brown dorsally, the ventral pleurites more yellowish. Halteres [illegible], the base of stem restrictedly pale. Legs with the coxæ and trochanters yellowish brown; femoral bases pale, the remainder dark brown; tibiæ entirely brownish black; tarsi snowy white, with nearly the proximal half (fore legs) to third (posterior legs) brownish black. Wings (Plate 1, fig. 22) with a strong blackish suffusion on more than the cephalic half, the posterior portion, including cells R_5 to 2d A, more grayish; veins dark brown. Venation: Sc_1 ending opposite or shortly before proximal end of R_2; Sc_2 shortly before fork of Rs.

Abdominal tergites, including hypopygium, black; basal sternites obscure yellow.

MINDANAO, Surigao Province, Diuata Mountains, April 10, 1931 (*Dayag*); holotype, male; Mount Cantugas, March 19, 1931 (*Dayag*); allotype, female; paratypes, several of both sexes, with the types.

Trentepohlia (*Anchimongoma*) *beata* is very distinct from the only other Philippine species of the subgenus, *T.* (*A.*) [illegible] Alexander, in the pattern of the legs, with no white on the femoral tips of tibial bases. The species is more nearly allied to the subgenotype, *T.* (*A.*) *simplex* (Brunetti), of India, differing conspicuously in the coloration of the wings and legs.

base of abdomen; flagellar segments cylindrical, with verticils shorter than the segments. Head black.

Mesonotum almost uniformly dark brown. Pleura dark brown dorsally, the ventral pleurites more yellowish. Halteres black, the base of stem restrictedly pale. Legs with the coxæ and trochanters yellowish brown; femoral bases pale, the remainder dark brown; tibiæ entirely brownish black; tarsi snowy white, with nearly the proximal half (fore legs) to third (posterior legs) brownish black. Wings (Plate 1, fig. 22) with a strong blackish suffusion on more than the cephalic half, the posterior portion, including cells R_5 to 2d A, more grayish; veins dark brown. Venation: Sc_1 ending opposite or shortly before proximal end of R_2; Sc_2 shortly before fork of Rs.

Abdominal tergites, including hypopygium, black; basal sternites obscure yellow.

MINDANAO, Surigao Province, Diuata Mountains, April 10, 1931 (*Duyag*); holotype, male; Mount Cantugas, March 19, 1931 (*Duyag*); allotype, female; paratypes, several of both sexes, with the types.

Trentepohlia (*Anchimongoma*) *beata* is very distinct from the only other Philippine species of the subgenus, *T.* (*A.*) *apoicola* Alexander, in the pattern of the legs, with no white on the femoral tips of tibial bases. The species is more nearly allied to the subgenotype, *T.* (*A.*) *simplex* (Brunetti), of India, differing conspicuously in the coloration of the wings and legs.

ILLUSTRATIONS

[Legend: *a*, ædeagus; *b*, basistyle; *dd*, dorsal dististyle; *g*, gonapophysis; *id*, inner dististyle; *od*, outer dististyle; *p*, phallosome; *s*, 9th sternite; *t*, 9th tergite; *vd*, ventral dististyle.]

PLATE 1

FIG. 1. *Dolichopeza (Nesopeza) abdita* sp. nov., wing.
2. *Dolichopeza (Nesopeza) inornatipes* sp. nov., wing.
3. *Limonia (Limonia) monilis* sp. nov., wing.
4. *Limonia (Limonia) thetica* sp. nov., wing.
5. *Limonia (Limonia) semantica* sp. nov., wing.
6. *Limonia (Limonia) trigonella* sp. nov., wing.
7. *Limonia (Libnotes) astuta* sp. nov., wing.
8. *Limonia (Libnotes) quadriplagiata* sp. nov., wing.
9. *Limonia (Libnotes) henrici* sp. nov., wing.
10. *Limonia (Alexandriaria) nathalinæ* sp. nov., wing.
11. *Limonia (Alexandriaria) tecta* sp. nov., wing.
12. *Helius (Helius) costosetosus* sp. nov., wing.
13. *Helius (Helius) devinctus* sp. nov., wing.
14. *Ula auritarsis* sp. nov., wing.
15. *Limnophila (Limnophila) petulans* sp. nov., wing.
16. *Pilaria (Eupilaria) auranticolor* sp. nov., wing.
17. *Gonomyia (Lipophleps) sagittifera* sp. nov., wing.
18. *Gymnastes (Paragymnates) hylæa* sp. nov., wing.
19. *Teucholabis (Teucholabis) rutilans* sp. nov., wing.
20. *Taiwanina mindanica* sp. nov., wing.
21. *Trentepohlia (Mongoma) alboterminalis* sp. nov., wing.
22. *Trentepohlia (Anchimongoma) beata* sp. nov., wing.

PLATE 2

FIG. 23. *Dolichopeza (Nesopeza) abdita* sp. nov., male hypopygium.
24. *Dolichopeza (Nesopeza) inornatipes* sp. nov.. male hypopygium, details.
25. *Dolichopeza (Nesopeza) bicornigera* sp. nov.. male hypopygium. details.
26. *Dolichopeza (Nesopeza) ridibunda* sp. nov., male hypopygium, details.
27. *Limonia (Limonia) thetica* sp. nov., male hypopygium.
28. *Limonia (Limonia) semantica* sp. nov., male hypopygium.
29. *Limonia (Libnotes) quadriplagiata* sp. nov., male hypopygium.
30. *Limonia (Alexandriaria) nathalinæ* sp. nov., male hypopygium.

PLATE 3

FIG. 31. *Limonia (Alexandriaria) tecta* sp. nov., male hypopygium.
32. *Helius (Helius) costosetosus* sp. nov., male hypopygium.
33. *Helius (Helius) devinctus* sp. nov., male hypopygium.
34. *Limnophila (Limnophila) petulans* sp. nov., male hypopygium.
35. *Pilaria (Eupilaria) auranticolor* sp. nov., male hypopygium.
36. *Gonomyia (Lipophleps) sagittifera* sp. nov., male hypopygium.
37. *Gymnastes (Paragymnastes) hylæa* sp. nov., male hypopygium.
38. *Teucholabis (Teucholabis) rutilans* sp. nov., male hypopygium.

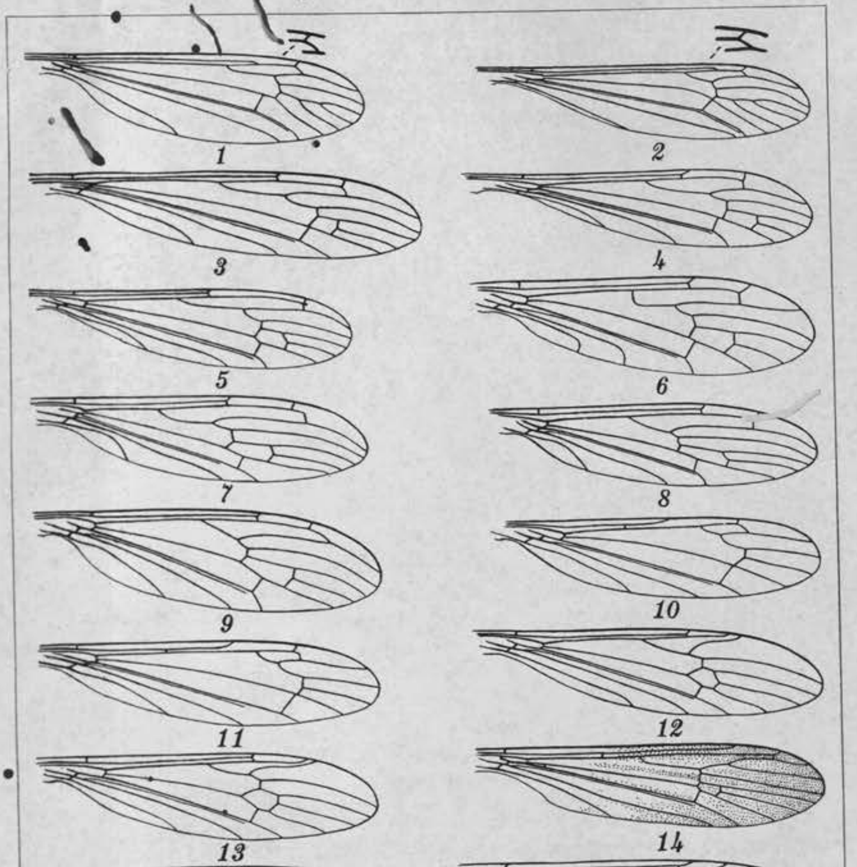


PLATE 1.

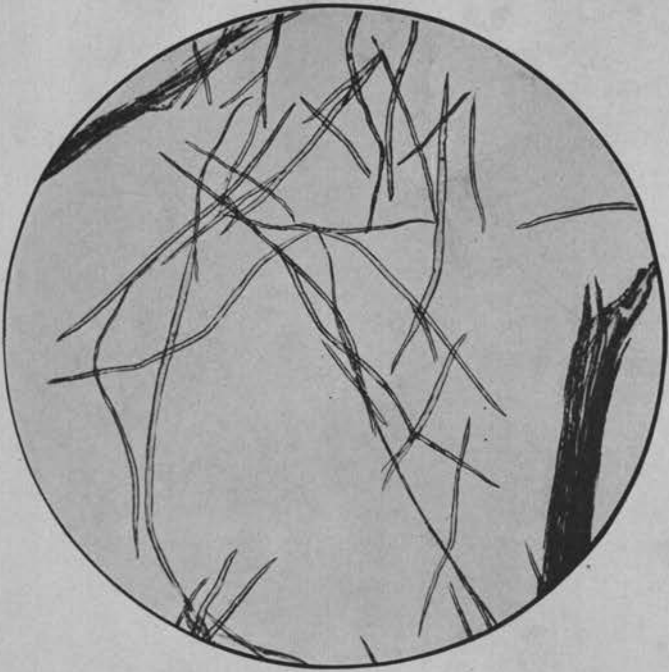


FIG. 1.

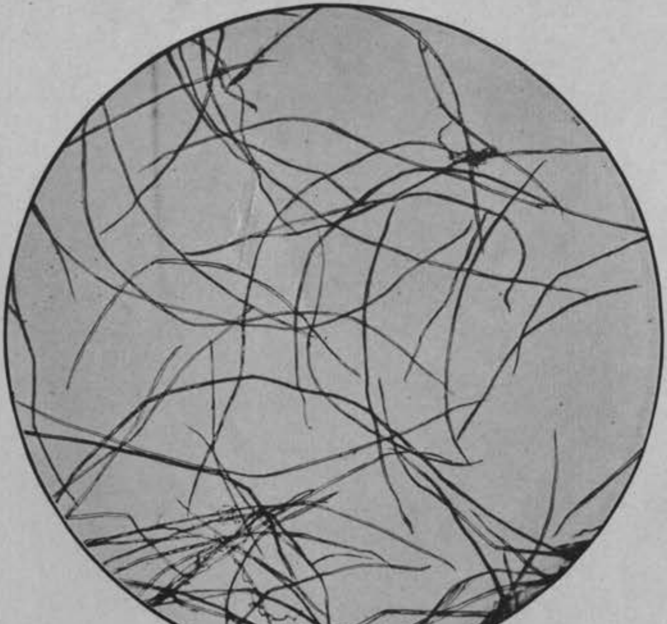
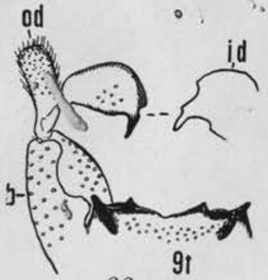


FIG. 2.

PLATE II.



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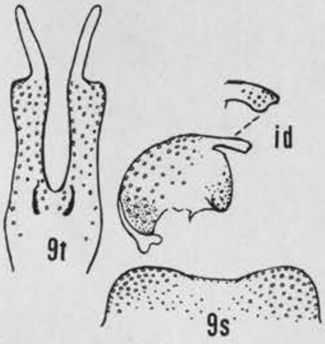

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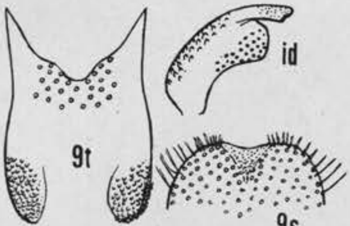

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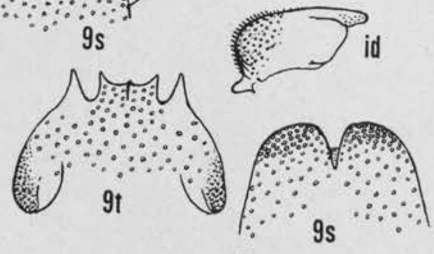

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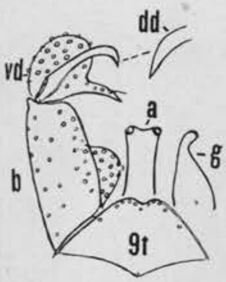

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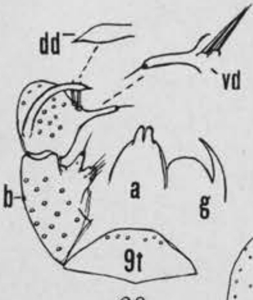

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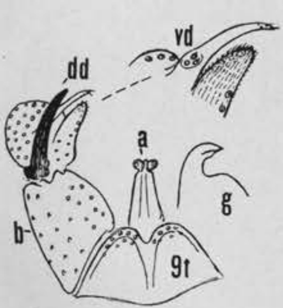

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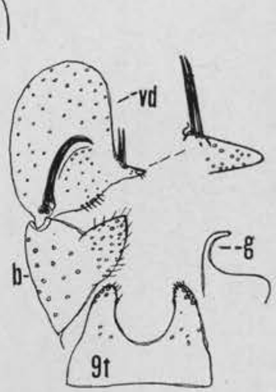

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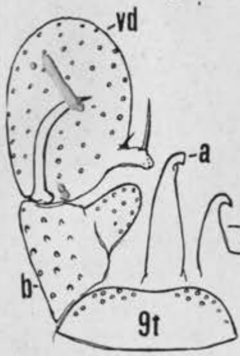
PLATE 2.



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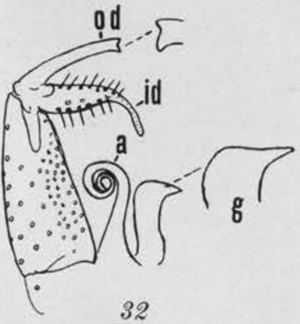

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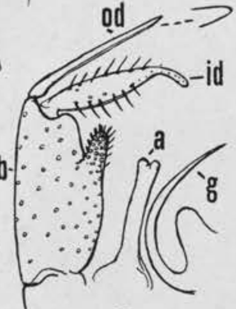

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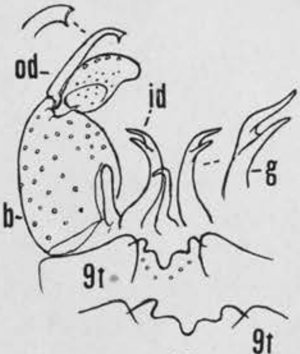

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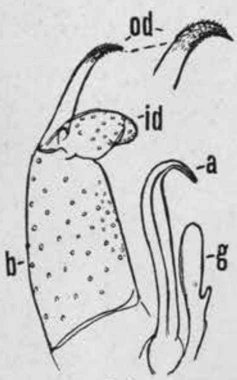

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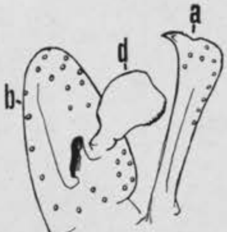

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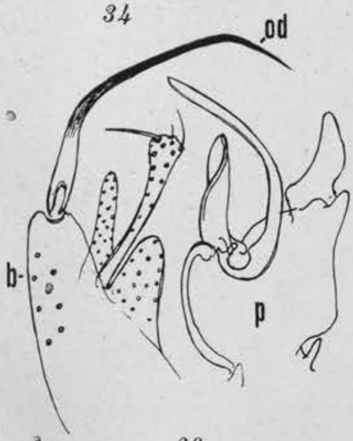

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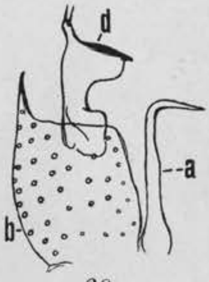

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PLATE 3.

TWO NEW CONIFER-INFESTING SCALE INSECTS FROM JAPAN

By INOKICHI KUWANA

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THREE TEXT FIGURES

ASPIDIOTUS MAKII sp. nov.

Scale of female.—Elongate oval, convex; white or dirty white. Exuviæ subcentral, orange yellow; usually covered with a white waxy substance. Ventral scale very thin; when removed the insect leaves a white patch on the host. Length, about 1.5 millimeters; width, about 1.

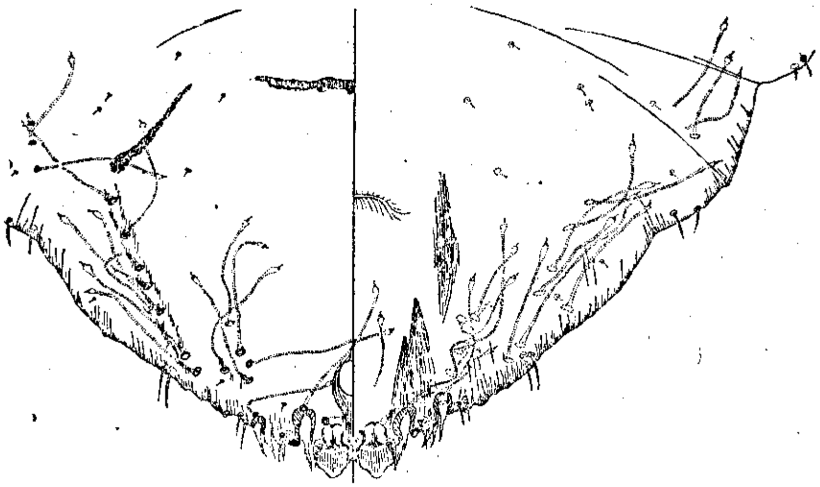

FIG. 1. Pygidium of *Aspidiotus makii* sp. nov.

Scale of male.—Similar to that of the female, elongate, white, subtransparent. Exuvia a little to one side, orange yellow. Length, about 1.3 millimeters; width, 0.7.

Body of female.—Nearly circular, narrowing towards posterior end. Mouth parts large, well chitinized, rostral loop very long. Antennæ rudimentary, conical, single jointed with a long spine. Spiracles rather large, with no parastigmatic gland orifice.

Pygidium of female.—Rather large, margin round. One pair of lobes, prominent, broader than long to a greater or less extent, notched on each side; no indication of second and third lobes. Two distinct lateral incisions on each side of the margin, bounded by conspicuous thickenings, the inner the larger. Plates small, rather inconspicuous, two between the median lobes, which are much shorter than the lobes and fimbriated at extremity; four or five along the margin, laterad of the median lobe, they are rather long and usually laterally fimbriated. Spines strongly developed, as shown in the figure. Paragenital gland orifice wanting. Dorsal gland orifices fewer, slender, more or less regularly arranged in three series. Transverse basal thickening fairly developed, laterals separated from the central line and robust. Apical ventral chitinization weakly developed. Anal opening large and nearer to the margin than the base of the pygidium.

Habitat.—On *Pinus luchuensis* (Ryukiu matsu). Found by Mr. Yoshitada Maki, of the Kagoshima Sugar Experiment Station, in Wada-mura, Amami-Oshima, December 27, 1930.

This new scale insect is allied to *Aspidiotus uvæ* Comstock and *A. ulmi* Johnson in the pygidial structures in general, but differs from them in the shape of the median lobes, and is readily distinguishable from these two species. The species is named for Mr. Maki, the collector of the type specimens.

ASPIDIOTUS PSEUDOMEYERI sp. nov.

Scale of female.—Elongate oval or irregularly circular, convex. Dull greenish yellow; older specimens dirty pale yellow with posterior margin brown. Mouth parts large, well developed, rostral loop short. Spiracles with no parastigmatic gland orifice. Antennæ minute, conical with a spine.

Pygidium of female.—Broadly round, not heavily chitinized. Two pairs of lobes prominent, well chitinized; median pair largest, apices round with no notch on either side; second pair much smaller but the same general shape as the median; a third pair is present, conical, often very small and obscure. Incisions moderate, very distinct. Paraphysis practically wanting. Plates broad, branched, extending but slightly beyond the lobes, two between the median lobes, two between the median and second lobes, and three between the second and third lobes. Spines usual, as shown in the figure. Anal opening large, circular, placed nearer to the margin than the base of the pygidium. Dorsal pores rather large, not regularly arranged.

Paragenital gland orifices in four groups, each group with few pores as shown in the following examples:

4–4 4–3 3–2
2–2 2–2 2–1

Basal thickenings represented by two median transverse rods and the widely separated oblique lateral thickenings; ventral thickenings normal, but slightly chitinized.

Habitat.—On *Juniperus chinensis* (Ibuki or biyakushin). Collected by Mr. T. Mayeda, of Yokohama, March 25, 1930. Previously collected by the writer in the same place.

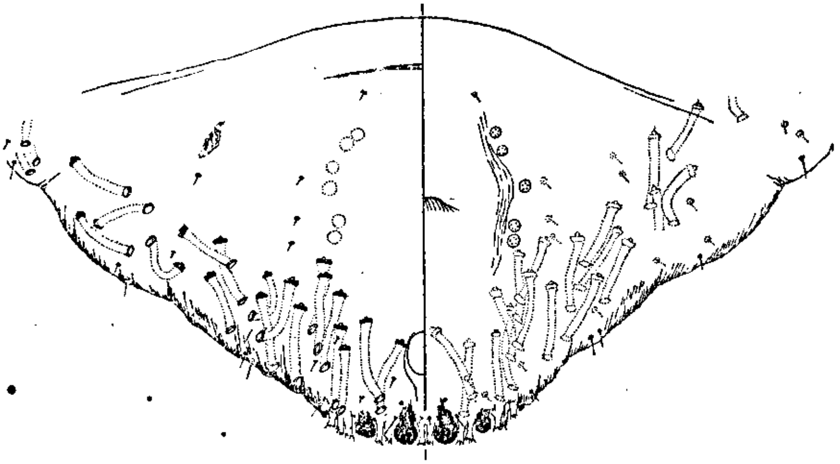

FIG. 2. Pygidium of *Aspidiotus pseudomeyeri* sp. nov.

This new scale insect is very closely allied to *Aspidiotus meyeri* Marlatt, but diverges from the latter in the following structural features:

A. meyeri.	*A. pseudomeyeri.*
With a distinct notch on either side of the median and second lobes.	None.
With four of five branched plates following the third lobe.	None.

While the writer was working with the conifer-infesting *Aspidiotus*, he had the opportunity of examining *Aspidiotus abietis* (Schr.) from Mississippi and from Italy. In going through Japanese forms and comparing these two foreign specimens with them, the writer came to the conclusion that *Aspidiotus abietis* is not found in Japan. At the same time he has noted that the American specimen, although named *Aspidiotus abietis*, is *Aspidiotus pini* Comstock instead. Some writers have placed *A.*

pini Comstock as a synonym of *A. abietis* (Schr.), but they are distinct species and can be easily separated by the following characters as shown in the figures:

A. pini.	*A. abietis.*
About one-third of the distance from the third lobe to the penultimate segment is a lobe of the lateral margin of the pygidium, about the size of the third lobe.	No such lobe on the margin.
Dorsal pores are very numerous.	Dorsal pores are not so numerous.

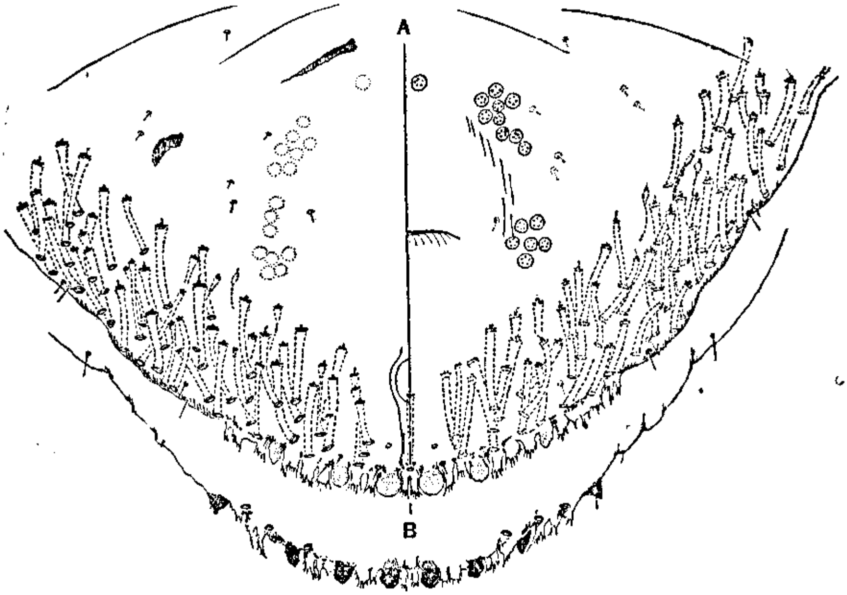

FIG. 3. *a*, Pygidium of *Aspidiotus abietis* (Schr.); *b*, margin of pygidium of *Aspidiotus pini* Comstock.

ACKNOWLEDGMENT

The writer's thanks are due to Dr. Harrold Morrison, United States Bureau of Entomology, Washington, D. C., for his courtesy in examining specimens, and for his assistance in their determination. The writer is also greatly indebted to Dr. F. Silvestri, of Italy, and to Mr. L. E. Myers, of Mississippi, who kindly furnished material for comparative study.

Tokyo, Japan, October 6, 1931.

ILLUSTRATIONS

TEXT FIGURES

FIG. 1. Pygidium of *Aspidiotus makii* sp. nov.
2. Pygidium of *Aspidiotus pseudomeyeri* sp. nov.
3. *a*, Pygidium of *Aspidiotus abietis* (Schr.); *b*, margin of pygidium of *Aspidiotus pini* Comstock.

THE GENUS ACLERDA IN JAPAN, INCLUDING FORMOSA

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FOUR TEXT FIGURES

INTRODUCTION

The present paper is made up of descriptions and notes on the known Japanese species of the genus *Aclerda,* subfamily Aclerdinæ, family Coccidæ, of which there are three in all. One is described here for the first time and the two other species are redescribed from new material collected by the writer and his assistants.

The writer's thanks are due to Messrs. E. E. Green and R. Newstead, of England, and to Mr. T. V. Nam Ayyar, of South India, for their courtesy in supplying the writer with material for comparative study. The writer's thanks are also due to Mr. Ryoichi Takahashi, of the Government Institute of Formosa, for presenting him with a species from the island.

Genus ACLERDA Signoret

The body of the adult female is thick, fleshy, almost soft, more or less hemispherical oval; creamy white with dark brown caudal end. Unprotected portions of the body covered with closely felted white wax, and those portions, dorsal and ventral, in contact with the food plant covered with a white mealy secretion.

Antennæ represented only by minute setiferous tubercles. Mouth parts well back from the front. Thoracic spiracles conspicuous, no stigmatic cleft nor specialized stigmatic spines. Legs wanting. The lateral margins of the body are usually covered with tuberculate setæ. Anal segment characterized by an undivided median plate enclosed in a somewhat deep cleft. Anal tube ending in many long spines (in the case of *A. biwakoensis* the spines are very short).

All the known larvæ are similar in their excessively elongated form. The margins of the body are nearly parallel, with a regular series of short, blunt spines. Antennæ and legs well developed. Anal lobes not prominent, each with a long hair.

Usually found beneath the leaf sheaths or on roots of various gramineous plants.

Three known species in Japan, may be separated by the following key:

Key to the species of Aclerda.

a^1. Anal plate circular with apex distinctly cleft	*A. tokionis.*
a^2. Anal plate with apex never cleft.	
b^1. Anal plate broadly round	*A. biwakoensis.*
b^2. Anal plate triangular	*A. takahashii.*

DESCRIPTION OF SPECIES

ACLERDA TOKIONIS (Cockerell).

Sphaerococcus (Pseudolecanium) tokionis COCKERELL, Psyche 7; Suppl. 1 (1896) 19; U. S. Dept. Agr., Technical Series, Bull. 4 (1896) 49.

Pseudolecanium tokionis (Cockerell), COCKERELL, Proc. Acad. Sci. Philadelphia (1899) 263; KUWANA, Proc. Cal. Acad. Sci. III 2 (1901) 403; 3 (1902) 57.

Aclerda tokionis (Cockerell), FERNALD, Cat. Coc. World (1903) 211; KUWANA, Imperial Agr. Exp. Sta. Japan Bull. 1 (1907) 117; KUWANA, Nippon Kaigaramushi Zusetsu 2 (1917) 44; FERRIS, Standford Univ. Biol. Ser. 1, No. 1 (1920) 133; TEAGUE, Annals Ent. Soc. Am. 18 (1925) 439; KUWANA, Oyo-Dobutsugaku Zasshi, No. 3, 3 (1931) 231.

Adult female.—Usually broadly oval in outline. Color of the body at first milky white and soft with the postabdominal margin castaneous, later the body becomes tinged with pink, and the castaneous area greatly extended with the deposition of denser chitin until finally the whole of derm may become hardened, and of a reddish brown color (dried specimens dark brown). The unprotected part of the body covered with white waxy filaments, and those portions, dorsal and ventral, in contact with the food plant with a white mealy secretion.

Antennæ very minute, consisting of one joint, conical, with a number of rather strong spines. Eyes apparently wanting. Mouth parts small, set well back from the front, and placed immediately below the anterior pair of spiracles; rostral loop very short; mentum monomerous. Spiracles large and prominent, the aperture of each with a crowded group of parastigmatic glands in a densely chitinized border. Legs wanting.

Posterior end of the abdomen with the wrinkling at the extreme margin of the body. Anal extremity with a distinct cleft.

Anal plate nearly circular with a broad notch in the posterior margin, bearing on each side of the margin about five hairy spines, and covering the extremity of the anal tube. Anal tube terminating in many very large, prominent spines that form a ring.

Posterior margin in the vicinity of the anal cleft with a number of rather long hairy spines. Marginal tuberculate setæ or spines rather small, bluntly pointed, strongly fusiform, forming a narrow zone which extends to the margins of the anal cleft. Dermal pores occur sparsely all over the body, but are more numerous in the marginal area. These are in two forms, one short and stout, the other small and slender. Lying in the area of tuberculate spines is a zone of multilocular pores, these being lacking on the anterior portion beyond the posterior pair of spiracles.

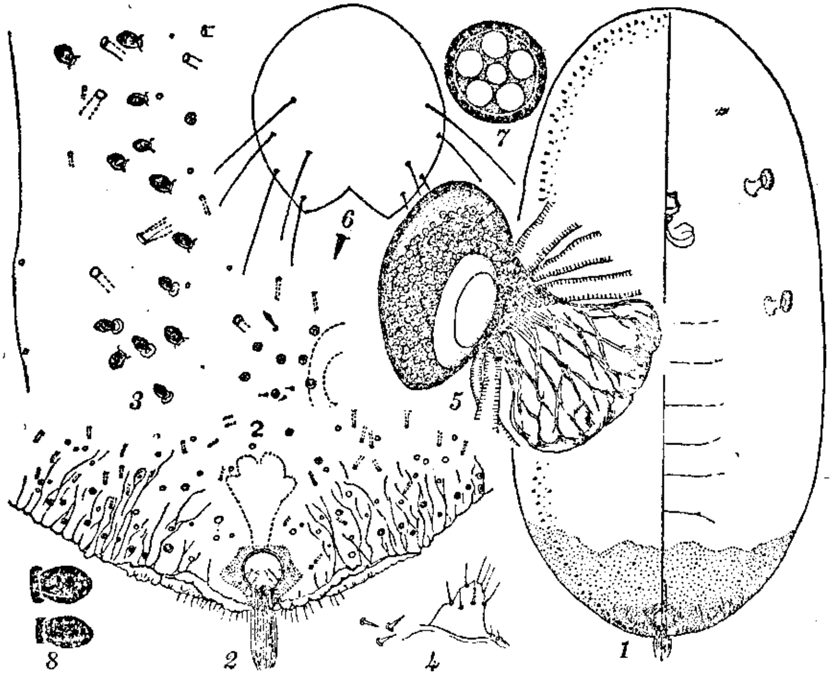

FIG. 1. *Aclerda tokionis* (female); *1*, adult female; *2*, abdominal end; *3* and *8*, margin with spines; *4*, antenna; *5*, spiracle; *6*, anal plate; *7*, multilocular pore. (All figures greatly enlarged.)

Length, 4 to 6 millimeters; width, 2 to 3.

Adult male.—Elongate, narrow. General color pale pink; two basal joints of antennæ pink, other joints of antennæ and legs pale brown; ocelli black; wings slightly tinged with brown. Head nearly round, pointed in front. Ocelli four, two dorsal

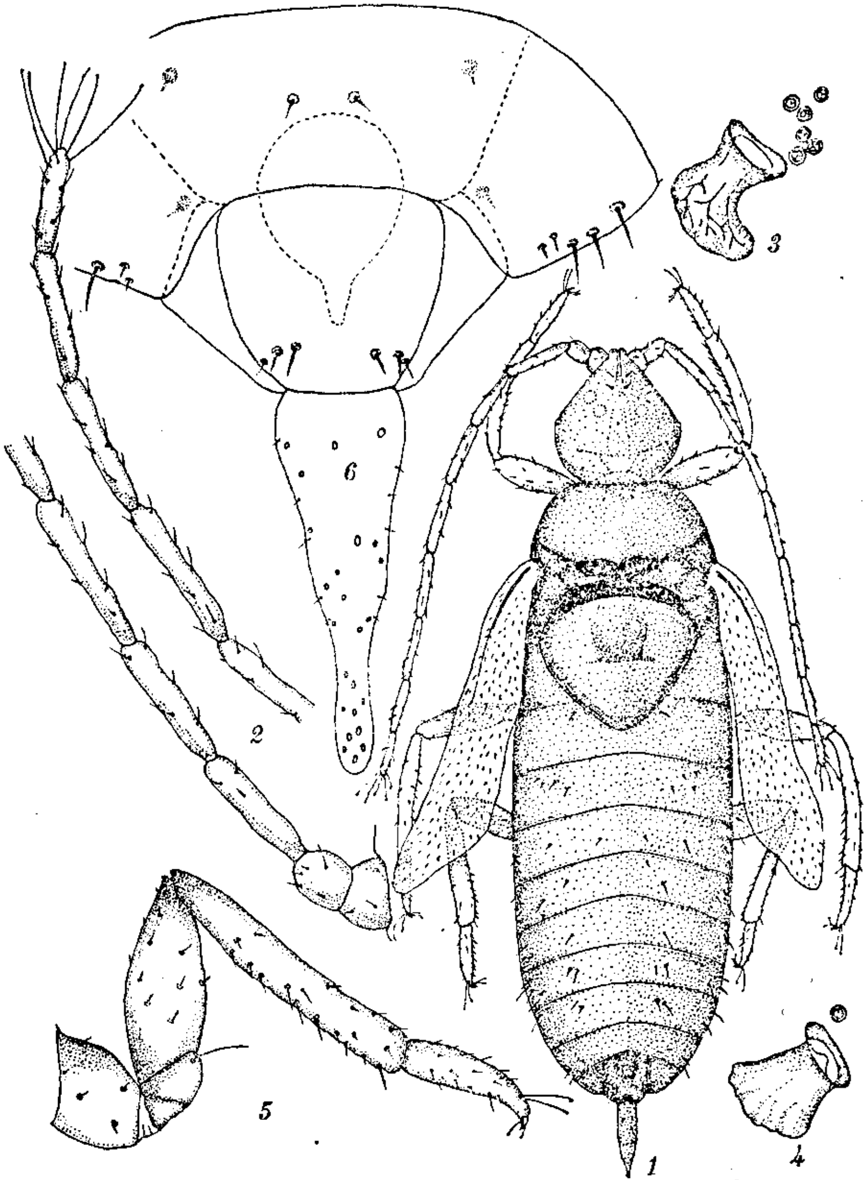

FIG. 2. *Aclerda tokionis* (male); *1*, adult male (dorsal view); *2*, antenna; *3*, anterior spiracle; *4*, posterior spiracle; *5*, hind leg; *6*, abdominal end. (All figures greatly enlarged.)

and two ventral; eyes minute, rudimentary, lateral. Antennæ ten-jointed, slender; first joint very short and stout; second somewhat longer than the first; other joints more slender and nearly equal in length, except the two terminal joints, which are somewhat shorter; each joint with scattered fine hairs, each almost as long as the joint itself. Thoracic region slightly narrower than the abdomen; prothorax short, mesothorax large and well developed, notal plate of mesothorax small; scutellum large and prominent, metathorax shorter than mesothorax. Wings large and narrow, rather thick, reaching about to the fourth abdominal segment, covered with minute hairs; halteres are not observed. Legs comparatively stout, covered with minute hairs; trochanter with a long hair and a well-developed transverse fold; femur much shorter than tibia; tarsus about one-third the length of the tibia, and more or less tapering towards the extremity; claw short and curved; digitules on tarsus and claw fine hairs, knobbed apically, the tarsal pair much the longer. Spiracles rather small, the anterior pair with about six multilocular pores, with only one in the posterior pair. Abdomen oval in shape, slightly wider than thorax, tapering towards the posterior end. The penis is formed from the ninth segment, and its enlarged base is enclosed slightly within the eighth segment. The penis is in the form of a long, slender, tapering stylus, slightly enlarged at the tip.

Length about 1.4 millimeters, width 0.4; length of wing, about 0.45; width, 0.10.

Male pupa.—Elongate in form, pinkish in color with antennæ and legs brown. Antennæ long and slender, reaching to the base of second legs, tapering towards the extremity. Wing case narrow and long, reaching to base of last legs.

Length, about 1.04 millimeters; width, 0.36.

Cocoon of male.—Elongate, subtransparent, white. Length, about 1 millimeter; width, 0.65.

Larva, first stage.—Oblong oval, narrow, segments distinct, sides nearly parallel. Pale yellowish in color with antennæ and legs pale. Antennæ rather short, six jointed; distinctly constricted between the joints; second joint shortest and the third longest; formula usually 3, 6, 5, 1, 4, 2. Legs long, three pairs subequal; femur slightly longer than tibia; claw slender, curved; digitules usual. Four capitate spines on dorsal side of caudal margin, the extremity with a deep cleft, and a small hair on each side. Caudal hairs rather long. Anal ring without hairs. A row of capitate spines all round the margin.

Length, about 1.5 millimeters; width, about 0.4.

Eggs.—Eggs in the body of the female elongate, pale yellow.

Habitat.—Beneath the leaf sheaths of the host plant, causing the sheaths to swell out at those points that cover the insects. This insect is ovoviviparous, but the eggs can be observed inside of the female. The young larvæ appear about early summer. The adult males are found in February. One generation a year, and the insect passes the winter in the stage of the adult female.

The host plants known at present in Japan are the following species of bamboo: *Yadakeya* (*Arundinaria*) *japonica* (yadake), *Pleisblastus chino* (Azuma sasa), and *Pleisblastus graminea* (Taimin chiku).

The scale insect is known from Japan, and the United States of America. In Japan it is commonest about Tokyo City; and, heretofore, it was collected in the following places beside Tokyo-fu: Fukushima, Saitama, Chiba, Kanagawa, Shizuoka, Aichi, Miye, and Gifu-ken.

Notes.—This insect was first discovered on bamboo in Tokyo by Mr. Otoji Takahashi, and was described by Prof. T. D. A. Cockerell in 1896. It was first found in California by the writer, on the campus of Leland Stanford Junior University in 1899. It is a native of Japan, and was probably introduced into California.

Although Mr. M. M. Teague has failed to note the marginal spines accompanied by a zone of stellate circular pores in the adult female of this species, the writer has seen the pores distinctly, around the margin caudate of about the posterior pair of spiracles to the caudal end; however, the pores are wanting in approximately the anterior half.

Since *Aclerda japonica* Newstead was found on *Arundinaria japonica* (Yadake), the writer has had some suspicion that it might prove to be this species, *Aclerda tokionis;* however, this problem cannot be solved without a comparative study of these two forms, which cannot be made at present.

ACLERDA TAKAHASHII Kuwana.

Aclerda japonica Takahashi (not of Newstead), TAKAHASHI, Research Inst. Government of Formosa, Dept. Agr. Report No. 48 (1930) 136.

Aclerda takahashii Kuwana (MS), KUWANA, Oyo-Dobutsugaku Zasshi No. 3, 3 (1930) 231.

Adult female.—Elongate oval in form with the posterior extremity more or less distorted in the old female; heavily chitinized. Color not observed. Antennæ very minute, consisting of a single joint carrying a number of rather strong spines. Mouth parts small, set well back from the frons, placed between the anterior pair of spiracles, rostral loop very short, mentum monomerous. Eyes obsolete. Spiracles much smaller when compared with *A. tokionis,* but the same in general shape, the aperture of each with numerous small pores. Legs wanting.

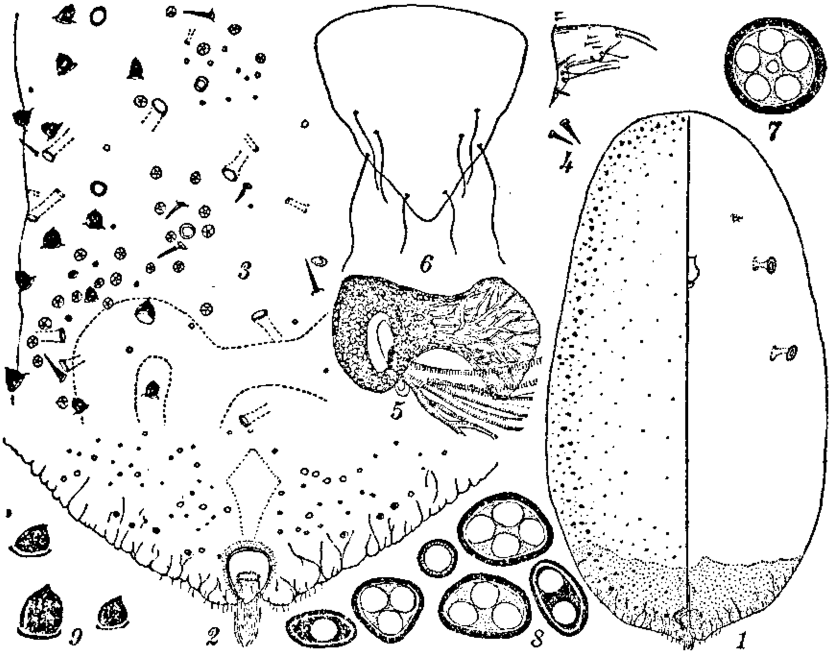

FIG. 3. *Aclerda takahashii* (female); *1,* adult female; *2,* abdominal end; *3* and *9,* margin with spines; *4,* antenna; *5,* spiracle; *6,* anal plate; *7* and *8,* multilocular pores. (All figures greatly enlarged.)

Anal extremity with a distinct anal cleft. Plate large, triangular in form, the apical extremity rounded, never cleft, with four rather long whiplike hairs on each side. Anal opening surrounded by many long hairs as in *A. tokionis,* and extending beyond the caudal end. Posterior margin in the vicinity of the anal cleft with many long, stout hairs. Marginal tuberculate spines or setæ in an irregular row that extends around the body except for a narrow region on each side of the anal cleft; the spines vary somewhat in size but all are of the same general shape, being broad at the base and terminating in a sharp point, which gives them a characteristic "acorn shape." Lying within the area of the tuberculated spines is a zone of multilocular pores, especially on the caudal margin up to about the anterior pair of spiracles. Dorsal pores scarce; they are in two types, large and small, the former more numerous.

Length, 3 to 6 millimeters; width, 2 to 4.

Habitat.—On *Saccharum officinarum* (sugar cane), Kori, Shinka, Formosa. Received a few mounted specimens from Mr. Ryoichi Takahashi, Taihoku, Formosa, in the summer of 1931.

The adult female of this insect resembles *A. tokionis* in general shape, but differs much in the shape of the anal plate, which is triangular with no cleft instead of round with a deep cleft. This triangular anal plate more nearly approaches that of *A. attinuata,* from which it can at once be separated by the arrangement of the hairs. Named in honor of Mr. R. Takahashi, who kindly furnished the writer with the material. This is the first description of the insect.

ACLERDA BIWAKOENSIS Kuwana.

Aclerda (?) *biwakoensis* KUWANA, Imperial Agr. Exp. Station of Japan, Bull. 1, No. 2 (1907) 187; COCKERELL, Can. Ent. 41 No. 2 (1907) 55; SANDERS, Cat. Rec. Desc. Cocc. 2, U. S. Dept. Agr. Tech. Ser. 16, pt. 3 (1909) 47.

Aclerda biwakoensis KUWANA, Nippon Kaigaramushi Zusetsu pt. 2 (1917) 76; TEAGUE, Ann. Ent. Soc. Am. No. 4, 18 (1925) 440.

Adult female.—Broadly oval to elongate in outline. Creamy color with a pinkish shade; when dried, it turns chestnut brown. Sides of the body nearly parallel and anterior end round, gradually broadening on the abdominal region with two shallow invaginations on each side of the body. Caudal extremity noticeably invaginated. More or less covered with a white waxy secretion.

Antennæ slightly conical, single jointed; bearing several strong spines. Mouth parts small, but well chitinized, rostral loop short, placed on the ventral surface, below the anterior pair of spiracles. Spiracles rather large, the aperture with a crowded group of parastigmatic glands in a densely chitinized border as in the preceding species. Eyes and legs wanting.

Anal cleft shallow, only visible on the dorsal surface, with a broadly round plate which bears six short spines on each side. Anal openings on the ventral side of this plate, which bears six very fine hairs in ring. Marginal tuberculate spines or setæ in a row, which extend around the body, increasing in number about the cleft. Spines, acorn shaped, but more or less curved at the tips. Numerous multilocular pores grouped about the spiracles. Derm with short tubular pores and fine hairs.

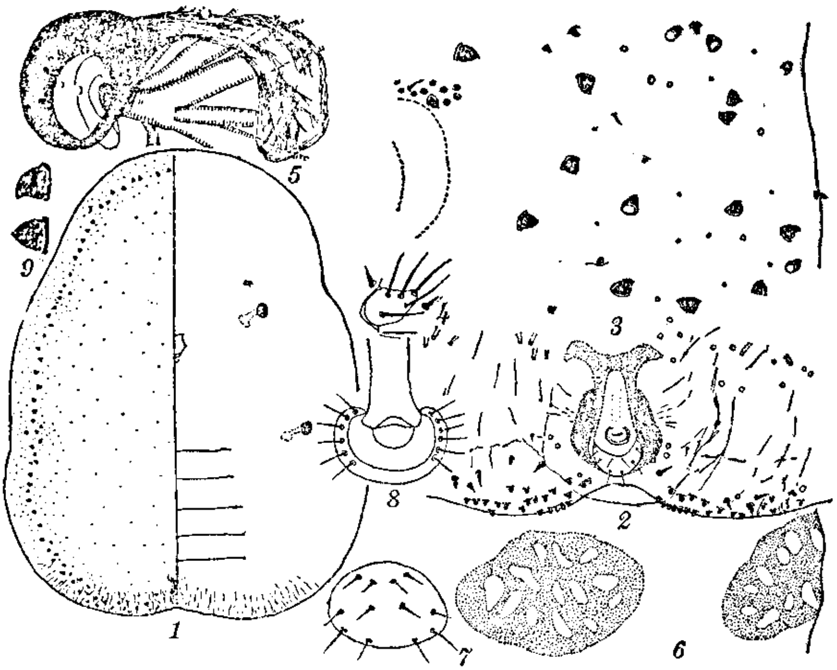

FIG. 4. *Aclerda biwakoensis* (female); *1*, adult female; *2*, abdominal end; *3* and *9*, margin with multilocular pores; *4*, antenna; *5*, spiracle; *6*, markings on dermes; *7*, anal plate; *8*, anal ring. (All figures greatly enlarged.)

Marginal area with irregularly round markings as shown in the figure.

Length, 6 to 8 millimeters; width, 5 to 6.

Habitat.—Beneath leaf sheaths of the food plant *Phragmites communis* (yoshi). This insect was originally described by the writer in 1917, from material personally collected on the host plant, which was growing on the shores of Lake Biwa, Shiga-ken. Since then the insect has been found by the writer and others in Fukui, Grifu, Aichi, Nara, and Miye-ken on the same host.

Notes.—On account of the anal cleft having a single plate, tuberculate or capitate spines along the margin of the adult female, and the shape of the newly hatched larva, the writer has for the present placed this insect in the genus *Aclerda;* however, it seems to him that as there is a great difference in the structure of the end of the anal tube, which bears very fine hairs instead of long, prominent ones, it might be advisable to establish a new genus.

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Tokyo, Japan, September 30, 1931.

ILLUSTRATIONS

TEXT FIGURES

FIG. 1. *Aclerda tokionis* (female); *1*, adult female; *2*, abdominal end; *3* and *8*, margin with spines; *4*, antenna; *5*, spiracle; *6*, anal plate; *7*, multilocular pore. (All figures greatly enlarged.)

2. *Aclerda tokionis* (male); *1*, adult male (dorsal view); *2*, antenna; *3*, anterior spiracle; *4*, posterior spiracle; *5*, hind leg; *6*, abdominal end. (All figures greatly enlarged.)

3. *Aclerda takahashii* (female); *1*, adult female; *2*, abdominal end; *3* and *9*, margin with spines; *4*, antenna; *5*, spiracle; *6*, anal plate; *7* and *8*, multilocular pores. (All figures greatly enlarged.)

4. *Aclerda biwakoensis* (female); *1*, adult female; *2*, abdominal end; *3* and *9*, margin with multilocular pores; *4*, antenna; *5*, spiracle; *6*, markings on dermes; *7*, anal plate; *8*, anal ring. (All figures greatly enlarged.)

ADDITIONS TO THE APHID FAUNA OF FORMOSA (HEMIPTERA)

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TWO TEXT FIGURES

Since the preparation of my revision of the Aphididæ of Formosa[1] several other species have been found in Formosa, which are here dealt with. The type specimens will be preserved in the Department of Agriculture, Government Research Institute, Formosa. I express here my sincere thanks to Dr. A. Mordvilko, of Leningrad, for his kindness in identifying specimens.

MYZUS CIRCUMFLEXUS Buckton.

BUCKTON, Monog. Brit. Aphid. 1 (1876) 130; THEOBALD, Journ. Econ. Biol. 8 (1913) 116; VAN DER GOOT, Contr. Fauna Indes Neerl. 1, 3 (1917) 50; SWAIN, Univ. Calif. Pub. Tech. Bull. Ent. 3 (1919) 74; BLANCHARD, Physis 5 (1922) 213; THEOBALD, Plant Lice Brit. 1 (1926) 331; HORI, Hokkaido Agr. Expt. Sta. Rept. No. 23 (1929) 87; KNOWLTON, Can. Ent. 61 (1929) 10; NEVSKY, Monog. Aphid. Middle Asia (1929) 119.

Host.—*Smilax* sp.

Habitat.—Taihezan (altitude about 5,500 feet).

A few specimens were collected by the author May 21, 1931. New to the fauna of Formosa.

LACHNUS NIGRIPES sp. nov.

Wingless viviparous female.—Dark brown, somewhat shining. Antennæ dark brown. Cornicles black. Cauda black on the hind margin in specimens treated with potash. Abdomen without dusky patches, but with a broad dusky band in front of the cauda. Legs black, femora somewhat paler on both ends in specimens treated with potash. Body oval, broad, about 3.5 millimeters in length, with many setæ scarcely or slightly curved. Head nearly straight on the front, divided, short, with many setæ, which are somewhat curved and much shorter than the first antennal joint. Antennæ slender, about 1.9 millimeters in length, with many rather stiff setæ as long as those on the head;

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[1] Dept. Agr., Govt. Res. Inst. Formosa, Rept. 53.

first joint as long as the second; third as long as the fourth, fifth and sixth taken together, with 1 to 6 small circular sensoria in a row on the distal part; fourth with 2 to 4 similar sensoria; fifth slightly imbricated on the distal part, with a large circular sensorium; the relative length of joints about as follows: III—48, IV—17, V—20, VI—10. Eyes with small ocular tubercles. Rostrum reaching a little beyond the hind coxæ. Abdomen distinctly reticulated, with a large median rounded conical tubercle. Cornicles on large hairy cones. Cauda short, very broadly rounded, with many long hairs. Legs very long, covered with many stout setæ; hind tibiæ somewhat curved, tapering, much stouter than the antenna, about 4 millimeters in length; hind tarsi nearly as long as the fourth antennal joint.

Host.—*Quercus glauca,* attacking the shoot.

Habitat.—Shinten near Taihoku.

A few apterous forms were taken by me June 14, 1931. Differs from other species of the genus *Lachnus* (synonym *Pterochlorus* Baker) in the black legs, and is also characterized by the reticulated abdomen. The Japanese species recorded by me as *Pterochlorus roboris* Linn.[2] is different from the present species in the larger body and in possessing some small dusky patches on the abdomen.

CERATOVACUNA ARUNDINARIÆ sp. nov.

Wingless viviparous female.—Yellow, densely provided with short white wax secretion. Body oval, moderately convex on the dorsum, about 1.8 or 1.9 millimeters in length, with many groups of very large well-developed wax pores on the whole dorsum and sides; the pores chitinized on the margin, protruding, oval, subcircular, or triangular with rounded corners, separated, nearly subequal in size, with minute polygonal markings, those on the eighth abdominal segment sometimes a little smaller. Dorsum somewhat chitinized on the areas of wax pores, without not-well-developed wax pores. Head and pronotum fused together, with some rather long fine hairs and areolations. Horns conical, pointed apically, expanded basally, stout, longer than wide, nearly as long as the first antennal joint, slightly diverging, somewhat curved upward, often with a slight constriction near the base, with a few short setæ. Eyes of three facets. Antennæ short, 4 jointed, about 0.25 millimeter long, with a few setæ; third joint expanded towards the distal end, with a circular sensorium as large as the facet of eye;

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[2] Aphididæ of Formosa, pt. 3, 115.

fourth joint a little shorter than the third. Rostrum reaching the middle coxæ. Thorax and abdomen with a few rather long, fine hairs. Cornicles very short, dusky, striate, nearly as large in diameter as the wax pore at the distal end, not on hairy cones. Cauda constricted at the base, wide, wider than the lobe of anal plate, with some bristles, of which two on the hind margin are longer. Anal plate bilobed, with some long bristles. Legs with some rather long fine hairs; front tibiæ nearly as long as the antenna; hind tibiæ about 0.4 millimeter long; hind tarsi nearly as long as or longer than the third antennal joint.

Host.—*Arundinaria* sp., attacking the lower side, especially the basal part, of leaf.

Habitat.—Taihezan (altitude about 5,500 feet).

Observed in abundance May 21, 1931. This species is allied to *Ceratovacuna silvestrii* Takahashi described from Yunnan, China, but is different from it in the shape of the frontal horns, as well as in the characters of the wax pores.

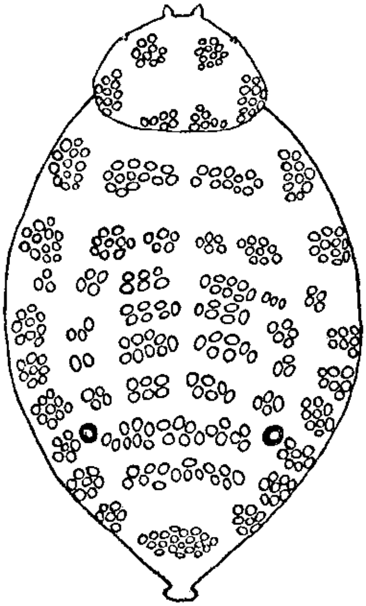

FIG. 1. *Ceratovacuna arundinariæ* sp. nov., adult female.

TRICHOREGMA NIPAE van der Goot.

Oregma nipae VAN DER GOOT, Contr. Fauna Indes Neerl. 1, 3 (1917) 208.

Host.—*Languas* sp. (Zingiberaceæ), attacking the lower side of leaf.

Habitat.—Urai.

Some apterous forms were taken by me September 6, 1931. Previously unknown from Formosa. The cornicles are on elevated cones which are provided with 6 or 7 long bristles, and the species is included in *Trichoregma* Takahashi. The Formosan specimens differ from the original description in that the horns are slightly shorter and the antennæ 4-jointed. In *Trichoregma* and the allied genera the antennæ are variable in the number of joints in the apterous form and the horns are also sometimes variable in length, and the above differences should not be specific, and now I adopt this name for the Formosan species on *Languas*.

THORACAPHIS SETIGERUS sp. nov.

Wingless viviparous female.—Black, with no secretion. Body elongate, oval, flattened, somewhat indented on the front margin, strongly chitinized, about 0.55 to 0.6 millimeter long, with about 5 very slight indentations on the side and 34 short stout spinelike submarginal setæ in a row along the whole margin; the setæ sharply pointed, slightly swollen about the middle, subequal in length, somewhat or not reaching beyond the body margin. Dorsum distinctly reticulated, without setæ. Head and thorax entirely fused together, occupying most of the body. Eyes of 3 facets. Antennæ and legs small, concealed under the body. Abdomen very small, not well defined from the thorax, but the eighth segment distinctly defined, rounded on the hind margin, with 4 setæ. Cornicles not discernible. Cauda and anal plate concealed; the former very small, wider than long, very slightly constricted basally; the latter divided.

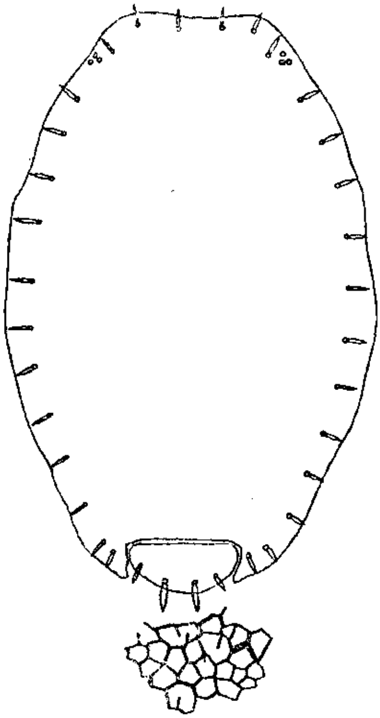

FIG. 2. *Thoracaphis setigerus* sp. nov., adult female and its dorsal reticulations.

Host.—*Quercus glauca*, attacking the lower side of leaf.

Habitat.—Urai, Shinten, Taihoku.

Many specimens were collected by me September 6, 1931. This species is characterized by possessing stout setæ along the margin of body, which is reticulated dorsally. The apterous forms are in very loose clusters. This form may be the aleyrodiform female of *Astegopteryx*.

THORACAPHIS KASHIFOLIÆ Uye.

Astegopteryx kashifoliæ UYE, Insect World, Gifu 28 (1924) 14.

Host.—*Quercus glauca*, attacking the upper side of leaf.

Habitat.—Shinten.

Many apterous females were collected by me June 14, 1931. Previously known only from Japan. The aleyrodiform female is wanting in reticulations and is not granular on the dorsum, but the cephalothorax is provided with 2 longitudinal furrows, which are somewhat diverging anteriorly and are connected by a transverse furrow on the hind ends, and with a short median furrow between the transverse furrow and the posterior margin of thorax.

FORDA FOLLICULARIA Pass.

Atti Soc. Ital. Sci. Nat. 3 (1861) 400.

Host.—*Paspalum* sp. (Gramineæ), attacking the root.

Habitat.—Kori.

New to the fauna of Formosa. Dr. A. Mordvilko has kindly compared the Formosan specimens with the European and Siberian ones, and writes me that the Formosan apterous forms have longer hairs on the body.

ILLUSTRATIONS

TEXT FIGURES

FIG. 1. *Ceratovacuna arundinariæ* sp. nov., adult female.
2. *Thoracaphis setigerus* sp. nov., adult female and its dorsal reticulations.

COMPOSITION OF PHILIPPINE SOY BEANS AND SOY-BEAN OIL

By AURELIO O. CRUZ and AUGUSTUS P. WEST

Of the Bureau of Science, Manila

Soy beans have been grown for many years in China, Korea, Japan, Formosa, and Indo-China. In recent years enormous quantities have been produced in Manchuria and exported to Europe. The beans are now also raised extensively in the United States. Some months ago we obtained a supply of soy beans raised in the Philippines. Our analyses of the Philippine soy beans and soy-bean oil showed that these products are very similar in composition to the soy beans and oil produced in other countries.

On account of their highly nutritive properties and protein content the addition of soy beans to the usual diet of Filipinos should be very desirable especially in outlying districts where meat is rather scarce and the food consists mostly of rice which is deficient in proteins and fats. The cultivation of soy beans in the Philippines would seem to offer promising prospects as a profitable and also very desirable industry.

During the past twenty years more than a thousand varieties of soy beans have been introduced into the United States from Far Eastern countries.[1] It seems that the bean can be grown successfully in any climate suitable for corn or cotton. However, experience has shown that some varieties do not grow so well in certain localities and, accordingly, a definite variety should be selected, by experimental growing, for each locality. Records of soy beans imported into the United States from China indicate that most Chinese villages have their own distinct varieties.

Soy beans grow well in combination with other farm crops. They also do well when grown in rotation with rice[2] and consequently make a very suitable crop for a country where rice is one of the principal agricultural industries. Like most other legumes soy beans are able to utilize the nitrogen of the air through the action of bacteria that live on the roots and develop nodules or tubercles.

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[1] Year Book, U. S. Dept. Agr. (1926) 678.

[2] Year Book, U. S. Dept. Agr. (1926) 673.

Soy beans may become infected with various leaf diseases which are manifest primarily in the form of leaf spots.[3] These may be controlled, however, by suitable methods.

Soy-bean oil is used largely in the manufacture of soaps, paints, varnishes, linoleum, enamels, lubricating oils, printing inks, waterproof goods, salad oils, and substitutes for rubber, lard, and butter. The oil cake remaining after the oil is extracted from the bean is a highly concentrated and nutritious food and is not only relished by all kinds of livestock but is also suitable for human consumption.

Soy-bean cultivation is not only profitable for the production of a commercially useful oil but also for various other purposes such as making hay, silage, and pasturage. During the last few years the number of companies in the United States manufacturing special soy-bean products has increased considerably. Soy beans are now made into breakfast foods, crackers, wafers, soy sauce, bean curd, and soy flour. Recipes for making soy-bean preparations are given in various publications.[4] Miss Maria Y. Orosa, of the Bureau of Science, has made excellent cakes, cookies, puddings, sauces, soups, custards, ice cream, and other tasty preparations from Philippine soy beans.

The nutritional value of soy beans depends upon the fact that they contain about 20 per cent of edible oil and have a very high protein content ranging from about 30 to 40 per cent. The protein of soy beans, unlike that of other vegetables, is similar to animal proteins and seems to fulfill all physiologic requirements.[5] Soy-bean protein appears to be quite as valuable as the casein of milk. These conclusions are based upon actual feeding experiments and not merely upon food values calculated from chemical analyses as the latter cannot be considered an adequate method for determining the nutritive efficiency of a given food. A food may have a high protein content and yet only a portion of the amino acids obtained from these proteins may have a highly nutritional value. According to Osborne and Campbell[6] the chief protein of soy bean is a globulin, glycinin. When hydrolyzed this protein yields amino acids comparable to those of cow's milk and the proportion of amino acids is not very different from that found in animal flesh.

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[3] Lehman, S. G., Journ. Agr. Research 36 (1928) 811. Piper, C. V., and W. J. Morse, The Soy Bean (1923) 280.

[4] Piper, C. V., and W. J. Morse, The Soy Bean (1923) 259. Orosa, M. Y., Soy beans as a component of a balanced diet and how to prepare them, Bur. Sci. Pop. Bull. 13 (1932).

[5] Daniels, A. L., and N. B. Nichols, Journ. Biol. Chem. 32 (1917) 95. Osborne, T. B., and L. B. Mendel, Journ. Biol. Chem. 32 (1917) 375.

Soy beans contain only traces of starch and so flours made from soy beans are naturally a valuable addition to the dietary of diabetics. The protein of soy flour, when thoroughly cooked, is about 91 per cent digestible and the carbohydrates about 94 per cent.

Milk made from soy beans is quite comparable to animal milk and has similar properties. Soy milk is used extensively throughout China for infant feeding. Soy milk has been found to be very useful in cases of summer diarrhœa and other intestinal disorders where human or cow's milk was not well tolerated.[7]

In addition to their high protein content, soy beans are also exceptionally nutritious in that they contain a fairly liberal supply of both the water-soluble and fat-soluble vitamins.[8]

Experiments carried out by Daniels and McClung[9] have shown that in cooking soy beans at a temperature of 120° or with an excess of sodium bicarbonate there was no great destruction of the antineuritic vitamin.

An excellent account and bibliography of the soy bean is given by Piper and Morse.[10]

Soy beans in the Philippines.—Just when soy-bean cultivation was first started in the Philippines is not known. For years casual plantings have been made but it is only in recent years that the cultivation has been seriously considered as an agricultural industry. Two of the most widely used foods manufactured from soy beans are soy-bean curd and soy sauce, known in the Philippines as "toyo." The Chinese have introduced and extended the use of these products throughout the

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[6] Osborne, T. B., and S. F. Campbell, Conn. Agr. Exp. Sta. Rep. 21 (1897) 374.

[7] Muggia, A., and E. Gasca, Gazz. Ospedali Clin. 42 (1921) 356.

[8] Daniels, A. L., and N. B. Nichols, Journ. Biol. Chem. 32 (1917) 96. Osborne, T. B., and L. B. Mendel, Journ. Biol. Chem. 32 (1917) 376.

[9] Journ. Biol. Chem. 37 (1919) 201.

[10] The Soy Bean, McGraw-Hill Co. (1923).

East. For years both of these preparations have been made by Chinese in the Philippines.

The first investigation carried out in the Philippines on the nutritive value of soy beans was made in 1912 by Gibbs and Agcaoili [11] of the Bureau of Science. They analyzed the soy-bean curd made in the Philippines and described briefly the local method of manufacture. They also pointed out the fact that soy beans serve as an important nitrogenous food in countries like China and Japan where rice is the principal article of diet and stated that the employment of this food was rapidly extending and its value becoming appreciated in other countries. They also mentioned the fact that large quantities were being imported into the Philippines from southern China, principally from Amoy and Hongkong. In a paper published a few months later Gibbs, Agcaoili, and Shilling [12] gave analyses of Philippine soy (toyo) sauce and also notes on the local manufacture of this product, which is a Chinese sauce of the Worcestershire type. A more-detailed account of the manufacture of soy sauce as prepared in the Philippines is given by Salazar.[13] Groff [14] gives an excellent description of the process as practiced in Canton, China.

An investigation of the nitrogen content of soy beans imported into the Philippines was made by Brill and Alincastre [15] of the Bureau of Science in 1917. Field tests on the growing of soy beans in the Philippines have been carried out by Layosa [16] who mentioned the fact that soy beans are used in China, Japan, and other countries as a substitute for meat. Experiments on seed selection for Philippine cultivation have been made by Maceda.[17] Prominent among others who have written technical articles suggesting the advisability of growing soy beans in the Philippines for their nutritive value may be mentioned Barrett,[18] Wester,[19] and Mendiola.[20]

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[11] Philip. Journ. Sci. § A 7 (1912) 47.

[12] Philip. Journ. Sci. § A 7 (1912) 383.

[13] Philip. Agr. 15 (1926) 219.

[14] Philip. Journ. Sci. 15 (1919) 307.

[15] Philip. Journ. Sci. § A 12 (1917) 127.

[16] Philip. Agr. and For. 6 (1918) 276.

[17] Philip. Agr. and For. 8 (1919) 92.

[18] Philip. Agr. Rev. 4 (1911) 594; 6 (1913) 348.

[19] The Food Plants of the Philippines, Philip. Bur. Agr. Bull. 39 (1925) 180.

[20] A Manual of Plant Breeding for the Tropics (1926) 230.

During recent years soy beans have been grown to a limited extent in the Philippines. According to Doctor Roxas, director of the Bureau of Plant Industry, 4,218 tons were produced in 1930.

In 1911 Barrett,[21] of the Philippine Bureau of Agriculture, wrote as follows:

> Now is the time for the Philippine Agriculturist to take up soy bean culture in earnest, and to develop it in the same way, even if not to the same degree, as our neighbors across the way have been doing for centuries.

Twenty years after this advice was given we find the Philippines are producing an annual yield of only a few thousand tons of soy beans although, as shown by experiments, this is a very suitable and appropriate crop for this country.

The diet of the Filipino masses consists largely of rice and is more or less deficient in fats, proteins, and vitamins, which are the principal nutritive constituents of soy beans. In order to familiarize the people with the food value of soy beans it would seem that some incentive, other than technical literature, is desirable to get the Filipinos really soy-bean minded. With this idea in view the Bureau of Science has recently taken steps to popularize the use of soy beans in the Philippines. Articles by members of the staff[22] have been published in the local papers. In these articles attention has again been called to the nutritive properties of the soy bean and the point particularly stressed was the suggestion to include soy beans as a regular part of the Filipino diet with the idea of supplying economically, to a considerable extent, the present deficiency. A ration consisting of about 20 per cent soy beans and 80 per cent rice has been suggested as a rather fairly balanced diet which should, of course, be supplemented by meat and fresh vegetables when convenient. At the recent Philippine carnival in Manila the division of food preservation of the Bureau of Science gave a display of food products made from soy beans. A bulletin[23] was also issued explaining how these foods may be prepared. During the coming year the Bureau of Science will send to the provinces, from the division of food preservation, assistants who will explain the value of soy beans as an addition to the usual Filipino diet and also demonstrate how to cook them. If we can popularize the use of soy beans and create a general demand for them agricultural development will naturally follow, with the result that the Filipinos will be better nourished on home-grown products.

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[21] Philip. Agr. Rev. 4 (1911) 594.

[22] Brown, W. H., Philip. Tribune (Oct. 4, 1931). Brown, W. H., and A. J. Hermano, Philip. Herald (Oct. 24, 1931).

[23] Orosa, M. Y., Soy Beans as a Component of a Balanced Diet and how to prepare them, Bu. Science Pop. Bull. 13 (1932).

EXPERIMENTAL PROCEDURE

The Philippine soy beans used in this investigation were grown at the College of Agriculture, Los Baños, and kindly presented to us by Dr. Nemesio B. Mendiola. The beans were ground to a fine powder and sieved to eliminate the coarser particles. The composition of Philippine soy beans compared with foreign soy beans and flour is given in Table 1.

TABLE 1.—*Composition of Philippine and foreign soy beans, straight grade flour, and soy-bean oil cake.*

Constituent.	Soy beans.		Straight grade flour.[b]	Philippine soy-bean oil cake.[c]
	Philippine.	Foreign.[a]		
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Moisture	4.95	13.88	9.93	5.88
Fat	20.07	17.85	0.92	4.99
Protein	39.08	37.87	13.74	46.45
Ash	5.25	4.26	0.52	6.24
Crude fiber	5.69	6.30	----------	6.77
Carbohydrates (by difference)	24.96	19.84	74.89	29.67
Total	100.00	100.00	100.00	100.00
Calories per kilo	4,492.00	4,026.00	3,719.00	3,585.00

[a] Average of four analyses of foreign soy beans, Holtz, E., Seifensieder Ztg. 56 (1929) 103, 112, 121, 130.

[b] Leach, A. E., and A. L. Winton, Food Inspection and Analysis (1920) 320, 322.

[c] Composition calculated.

As shown by the data the Philippine soy beans have a higher calculated food value than either the foreign beans or straight grade flour.

Soy-bean oil was prepared by extracting the ground seeds with ether. The oil was purified by treating successively with 2 per cent kieselguhr, suchar, and talcum powder. This treatment removes vegetable fibers and colloidal matter and produces a brilliantly clear dark yellow oil. The yield of oil is about 20 per cent.

The constants of this sample of Philippine soy-bean oil are given in Table 2.

TABLE 2.—*Physical and chemical constants of Philippine soy-bean oil.*

Specific gravity at $\frac{30^\circ}{4^\circ}$ C.	0.9153
Refractive index at 30° C.	1.4710
Iodine number (Hanus)	128.4
Saponification value	193.3
Unsaponifiable matter (per cent)	1.00
Acid value	1.5
Saturated acids, determined (per cent)	13.71
Unsaturated acids plus unsaponifiable matter, determined (per cent)	81.44
Saturated acids, corrected (per cent)	12.65
Unsaturated acids, corrected (per cent)	81.50
Iodine number of unsaturated acids	151.3

The saturated and unsaturated acids that occur as glycerides in Philippine soy-bean oil were separated by the lead-salt-ether method[24] in accordance with the suggestions of Baughman and Jamieson.[25] The results are recorded in Table 3.

TABLE 3.—*Separation of saturated acids from the unsaturated acids in Philippine soy-bean oil by the lead-salt-ether method.*

Experiment No.—	Oil used.	Unsaturated acids.	Saturated acids.	Unsaturated acids (determined).	Saturated acids (determined).	Unsaturated acids (corrected).[a]	Saturated acids (corrected).
	g.	*g.*	*g.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	12.0720	9.7831	1.7130	81.04	[b] 14.19	81.52	12.71
2	11.4586	9.3780	1.5147	81.84	[c] 13.22	81.47	12.59
Mean				81.44	13.71	81.50	12.65

[a] Unsaturated acids (unsaponifiable matter removed): Iodine number (Hanus), 151.3.
[b] Iodine number (Hanus), 15.8.
[c] Iodine number (Hanus), 7.2.

The unsaturated acids separated from soy-bean oil by the lead-salt-ether method were purified by removing the unsaponifiable matter in accordance with the procedure used for determining the unsaponifiable. The resulting soaps were acidified and converted again into the pure mixed unsaturated acids. These mixed acids were then treated with bromine and converted into their bromo-derivatives.[26] An ether-insoluble hexabromide was obtained thus showing the presence of linolenic acid. The precipitated hexabromide was washed with ether which was previously saturated with linolenic hexabromide and cooled to 0°. The hexabromide used in making the ether solution was prepared from raw linseed oil. The solubility of the hexabromide is decreased considerably by washing with a cold, ether solution saturated with hexabromide. From the weight of hexabromide obtained the percentage of linolenic acid corresponding to the hexabromide is then calculated. Knowing the percentage of linolenic acid (2.41) contained in the mixed unsaturated acids, the iodine number of these mixed acids (151.3), and also the iodine numbers of the individual unsaturated acids (linolenic, linolic, and oleic), the percentage of linolic and oleic acids contained in the mixture is readily calculated. The composition of the mixed unsaturated acids is given in Table 4. There are also included the calculated percentages of glycerides corresponding to these individual unsaturated acids.

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[24] Lewkowitsch. J., Chemical Technology and Analysis of Oils, Fats, and Waxes 1 (1921) 556.

[25] Cotton Oil Press 6 (1922) 41. Journ. Am. Chem. Soc. 42 (1920) 2398.

[26] Lewkowitsch, J., Chemical Technology and Analysis of Oils, Fats, and Waxes 1 (1921) 585.

TABLE 4.—*Percentage composition of the unsaturated acids of soy-bean oil and the glycerides corresponding to these acids.*

Acid.	Mixture of unsaturated acids.	Original oil.	Glycerides in original oil.
	Per cent.	*Per cent.*	*Per cent.*
Linolenic	2.41	1.96	2.05
Linolic	62.21	50.70	52.98
Oleic	35.38	28.84	30.14
Total	100.00	81.50	85.17

Saturated acids.—The saturated acids were separated from soy-bean oil by the lead-salt-ether method and esterified with methyl alcohol. The mixed acids were dissolved in methyl alcohol and saturated with dry hydrogen chloride gas. The mixture was then heated on a water bath (reflux) for fifteen hours, after which it was treated with water and the ester layer separated. The esters were dissolved in ether and the ethereal solution washed with sodium carbonate solution and afterwards with water. The ethereal solution was then dehydrated with anhydrous sodium sulphate, filtered, and the ether removed by distilling. The impure esters, which were yellow, were distilled under diminished pressure. A preliminary distillation was made, after which the esters were redistilled. Data on the distillation of the esters are given in Tables 5 and 6.

TABLE 5.—*First distillation of the methyl esters of the saturated acids; 112.5908 grams of esters distilled.*

Fraction.	Temperature.	Pressure.	Weight.
	°C.	*mm.*	*g.*
A	190–193	7.0	26.1210
B	193–198	6.5	31.9196
C	198–203	7.0	28.5858
D	203–211	7.0	15.6537
E	211–218	7.0	5.3657
Residue			4.73
Total			112.3758

TABLE 6.—*Second distillation of the methyl esters of the saturated acids; 112.3758 grams of esters redistilled.*

Fractions.		Temperature.	Pressure.	Weight.
From first distillation.	Second distillation.			
		°C.	*mm.*	*g.*
A	1	190–192	5.5	16.6378
B	2	192	5.5	26.6660
C	3	192–197	5.5	18.2780
D and E	4	197–201	5.5	18.2498
Residue	5	201–204	5.0	14.3940
	6	204–215	5.0	10.7818
	7	215–238	5.0	5.4490
	Residue			1.78
Total				112.2364

In Table 7, are given the analyses of fractions obtained in the second distillation of methyl esters. From the data (Table 7), there were calculated the amounts of the individual acids corresponding to the methyl esters contained in the various fractions. The results are recorded in Table 8 and were calculated in accordance with the methods outlined by Baughman and Jamieson in their investigations of Hubbard squash-seed oil[27] and also American cottonseed oil.[28]

In Table 9 is given the composition of the mixed saturated acids and the glycerides in the original sample of soy-bean oil corresponding to these acids.

[27] Journ. Am. Chem. Soc. 42 (1920) 156.

[28] Journ. Am. Chem. Soc. 42 (1920) 1197.

TABLE 7.—*Analyses of fractions obtained in the second distillation of the mixed methyl esters.*[a]

Fraction.	Iodine number.	Saponification value.	Mean molecular weight of mixed esters.	Composition of mixed esters.		Mean molecular weight of saturated esters.
				Saturated.	Unsaturated.	
				Per cent.	*Per cent.*	
1	6.22	206.6	271.5	95.68	4.32	270.6
2	6.92	203.9	275.1	95.20	4.80	274.2
3	9.90	202.8	276.6	93.13	6.87	275.4
4	15.91	199.2	281.6	88.96	11.04	280.1
5	21.90	195.4	287.1	84.80	15.20	285.8
6	26.94	190.0	295.3	81.30	18.70	295.4
7	20.81	180.1	311.5	85.56	14.44	314.5

[a] Calculated iodine number of unsaturated methyl esters was 144.1. Calculated saponification value of unsaturated methyl esters was 190.4.

TABLE 8.—*Saturated acids corresponding to methyl esters in each fraction.*

Fraction.	Acids.					
	Palmitic.		Stearic.		Arachidic.	
	Per cent.	*g.*	*Per cent.*	*g.*	*Per cent.*	*g.*
1	89.76	14.93	0.97	0.16		
2	77.70	20.72	12.64	3.37		
3	72.22	13.20	16.17	2.96		
4	54.82	10.00	29.68	5.42		
5	35.89	5.17	44.75	6.44		
6	7.98	0.86	69.46	7.49		
7			34.36	1.87	47.39	2.58
Residue[a]						1.70
Total		64.88		27.71		4.28

[a] Residue assumed to be methyl arachidate.

TABLE 9.—*Saturated acids.*

Acid.	Mixture of saturated acids.			Glycerides in original oil.
	Weight.	Composition.	Proportion in original oil.	
	g.	*Per cent.*	*Per cent.*	*Per cent.*
Palmitic	64.88	66.98	8.47	8.89
Stearic	27.71	28.60	3.62	3.78
Arachidic	4.28	4.42	0.56	0.58
Total	96.87	100.00	12.65	13.25

The composition of Philippine soy-bean oil is given in Table 10. There is also included for comparison the composition of a sample of soy-bean oil analyzed by Baughman and Jamieson.

As shown by the data the composition of Philippine soy-bean oil is very similar to that of the American oil.

TABLE 10.—*Composition of Philippine soy-bean oil compared with American soy-bean oil.*

Constituent.	Soy-bean oil.	
	Philippine.	American.[a]
Glycerides:		
Unsaturated acids—		
Linolenic	2.1	2.3
Linolic	53.0	51.5
Oleic	30.1	33.4
Saturated acids—		
Palmitic	8.9	6.8
Stearic	3.8	4.4
Arachidic	0.6	0.7
Lignoceric	---------	0.1
Unsaponifiable matter	1.0	0.6
Total	99.5	99.8

[a] Composition determined by W. F. Baughman and J. S. Jamieson, Journ. Am. Chem. Soc. 44 (1922) 2947.

In Table 11 is given the composition of Philippine soy-bean oil compared with other oils. As shown by the data these oils have a somewhat similar composition and consist principally of a mixture of glycerides of linolic, oleic, and palmitic acids.

TABLE 11.—*Comparison of Philippine soy-bean oil with other oils.*

Constituent.	Philippine oils.				American cotton-seed oil.[d]
	Soy-bean oil.	Rice oil (hambas).[a]	Kapok-seed oil.[b]	Peanut oil.[c]	
Glycerides of:	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Unsaturated acids—					
Linolenic	2.1	---------	---------	---------	---------
Linolic	53.0	27.7	29.3	27.0	41.7
Oleic	30.1	45.6	49.8	53.9	35.2
Saturated acids—					
Myristic	---------	0.2	0.5	---------	0.3
Palmitic	8.9	17.3	15.9	8.5	20.0
Stearic	3.8	1.8	2.3	3.6	2.0
Arachidic	0.6	0.7	0.8	3.4	0.6
Lignoceric	---------	0.7	---------	2.4	---------
Unsaponifiable matter	1.0	4.0	0.8	0.3	---------
Total	99.5	98.0	99.4	99.1	99.8

[a] Philippine rice oil (hambas), A. O. Cruz, A. P. West, and N. B. Mendiola, Philip. Journ. Sci. (in press).

[b] Philippine kapok-seed oil, A. O. Cruz and A. P. West, Philip. Journ. Sci. 46 (1931) 131.

[c] Philippine peanut oil, A. O. Cruz and A. P. West, Philip. Journ. Sci. 46 (1931) 199.

[d] American cottonseed oil, G. S. Jamieson and W. F. Baughman, Journ. Am. Chem. Soc. 42 (1920) 1197.

The determined iodine number of Philippine soy-bean oil was found to be 128.4 and the determined saponification value 193.3. The calculated iodine number is 123.3 and the saponification value 189.7. The iodine and saponification values calculated from the composition of the oil agree very closely with the determined values.

SUMMARY

Like the soy bean raised in other countries the Philippine soy beans are very nutritious on account of the fats, carbohydrates, and proteins which they contain.

The protein of soy beans, unlike that of other vegetables, is similar to animal proteins and seems to fulfill all physiologic requirements.

Delicious cakes, cookies, sauces, soups, milk, custard, ice cream, and other tasty preparations have been made from Philippine soy beans.

Philippine soy-bean oil consists principally of the glycerides of linolic, oleic, and palmitic acids. It also contains small quantities of linolenic, stearic, and arachidic acids.

In composition, Philippine soy-bean oil resembles rice oil, kapok-seed oil, peanut oil, and cottonseed oil in that all these oils consist principally of the glycerides of linolic, oleic, and palmitic acids, though in different proportions.

The diet of the Filipino masses consists largely of rice and is more or less deficient in fats, proteins, and vitamins, which are the principal nutritive constituents of soy beans. To supply the present deficiency it has been suggested to include soy beans as a regular part of the Filipino diet. A ration consisting of about 20 per cent soy beans and 80 per cent rice gives a fairly balanced diet which should, of course, be supplemented by meat and fresh vegetables when convenient.

STUDIES ON EXPERIMENTAL CREEPING ERUPTION IN THE PHILIPPINES[1]

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FIVE PLATES AND ONE TEXT FIGURE

In 1926, Kirby-Smith, Dove, and White(8) reported on the frequent occurrence in the South Atlantic and Gulf States of America of a type of creeping eruption caused by a larval nematode which, pending the discovery of the adult worm at the time, they named *Agamonematodum migrans*. Subsequently, Dove and White(3) succeeded in producing experimentally, a typical creeping eruption of the type that naturally occurs in those States, by the application of the third stage larvæ of *Ancylostoma braziliense* on the intact skin of human volunteers, and demonstrated beyond doubt, that the *Agamonematodum migrans* which they had previously found to be involved in the lesions of creeping eruption, is no other than the third stage larva of this dog and cat hookworm, which is a common parasite of these animals in those regions. Fulleborn(4) in 1927 also succeeded in producing experimentally a similar skin eruption by using the infective larvæ of the European dog hookworm, *Uncinaria stenocephala.* Attempts to produce creeping eruption in man with the third stage larvæ of *Ancylostoma caninum, Strongyloides,* and of certain other parasitic nematodes have been made, but so far the results have been negative. For some unknown reason the larvæ of *A. braziliense* and *U. stenocephala* tend to remain in the skin of man and therein make horizontal migrations, instead of promptly penetrating deeper and entering the blood vessels, as they do in hosts to which they are well adapted.

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[1] Read in part before the joint meeting of the Philippine Society of Parasitologists and the Los Baños Biological Club at Tungkong Manga, Bulacan, October 3, 1931. The writer is indebted to Dr. Hilario Lara, acting director of the School of Hygiene and Public Health, for the deep interest he has shown in this investigation.

Ancylostoma braziliense is a very common parasite of cats in the Philippines. Schwartz(14) states that all the hookworms he examined in a collection from cats in this country were of this species. Philippine dogs also harbor this species of hookworm. Chandler(1) states that out of 480 hookworms recovered from Philippine dogs, 37 per cent were *A. braziliense*. Another reservoir host of this hookworm in this country is man. Manalang (Chandler, 1929) found over 11 per cent of 136 hospital cases infested with this species. According to Dove and White(3) creeping eruption occurs more frequently in the Southern United States during the summer months, especially after rainy weather. Children and persons who, by the nature of their occupation, have more contact with the soil are more liable to suffer from this skin affection. While there seems to be every reason to suspect that creeping eruption, of the type that has been found to be caused by the third stage larva of *A. braziliense* in the Southern United States, is also present in this country, it is rather strange that not a single case of this skin disease has so far been reported by our practitioners here. Dove and White(3) state that this creeping disease has been encountered rarely among the Negroes, suggesting a kind of racial resistance or immunity against the invasion of the skin by the larvæ of this worm. It should be recalled that this race also manifests a certain degree of resistance against human hookworm infestation. The question that is naturally raised, then, is: Is the apparent absence of creeping eruption in this country due to a natural resistance of the Filipino skin to the invasion of the third stage larva of *A. braziliense?* An attempt to answer this question forms the principal object of this investigation. Experimental infestations have been made on two human volunteers with the aim in view of producing typical lesions of creeping eruption by the application of the third stage larvæ of *A. braziliense* on intact skin. Incidentally, various other phases of this parasitic skin disease which have been observed in the course of this work will also be presented.

HISTORICAL

In order not to confuse the subject of this investigation with various other similar skin diseases known to be caused also by other metazoan parasites, a brief history of the origin of the term "creeping eruption" is deemed not out of place here. For decades, the term "creeping eruption" has been adopted by common usage to designate a group of skin affections which have, as a common characteristic, a linear cutaneous eruption with an advancing end, believed to be caused by a migrating parasite. Among the first to use this term was Lee(10) who in 1874 encountered in England a skin disease characterized by an advancing linear lesion. Although he later encountered another case, he failed to find its cause; but from the nature of the eruption, he was led to believe that the "lesion was due to an active parasite or animalcule" migrating in the skin. At the second Dermatological Meeting in Vienna, Crocker(2) reported a similar case, but, also failing to find the causative organism, he expressed the belief that the creeping lesion was probably due to the "travels of some insect larva either in the epidermis or beneath it" and suggested the name "larva migrans" for the disease. Samson and Himmelstjerna(13) reported on a small "worm" which they found a number of times in the epidermis of the human skin, and which was later identified by Cholodkovsky, an entomologist, as larva of the horse botfly, *Gastrophilus*. Kane(7) described a similar case of skin eruption involving the larva of *Hypoderma bovis*. Similar reports from various parts of the world describing skin affections, which involved the migration in the skin of the larvæ of various species of flies, then followed in rapid succession. Naturally, as a result of these findings, creeping eruption was for a time generally considered as a myasis, until Leiper(11) in 1909, Tamura(15) in 1921, and Morishita(12) in 1924, incriminated *Gnathostoma siamense*, *Echinorhyncus sphærocephalus*, and *Gnathostoma hispidum*, respectively, (all of which are nematodes) as causative parasites also in the production of creeping eruption.

MATERIALS AND METHODS

The third-stage larvæ of *A. braziliense* that were used in the following infestation experiments were obtained by screening, with Baermann apparatus, and 8-day-old fæcal culture from a cat with a presumably pure infestation of this species of hookworm, as was subsequently shown by the examination of the worms which were recovered from the intestines at autopsy. Known numbers of the infective larvæ were incorporated in small amounts of sterile moist soil, and these were applied to the skin of the flexor surface of the arm for about twenty minutes. At the end of that time, the soil was wiped off and the infested area washed with saline solution. The arm was used so as to make observations on the progress of

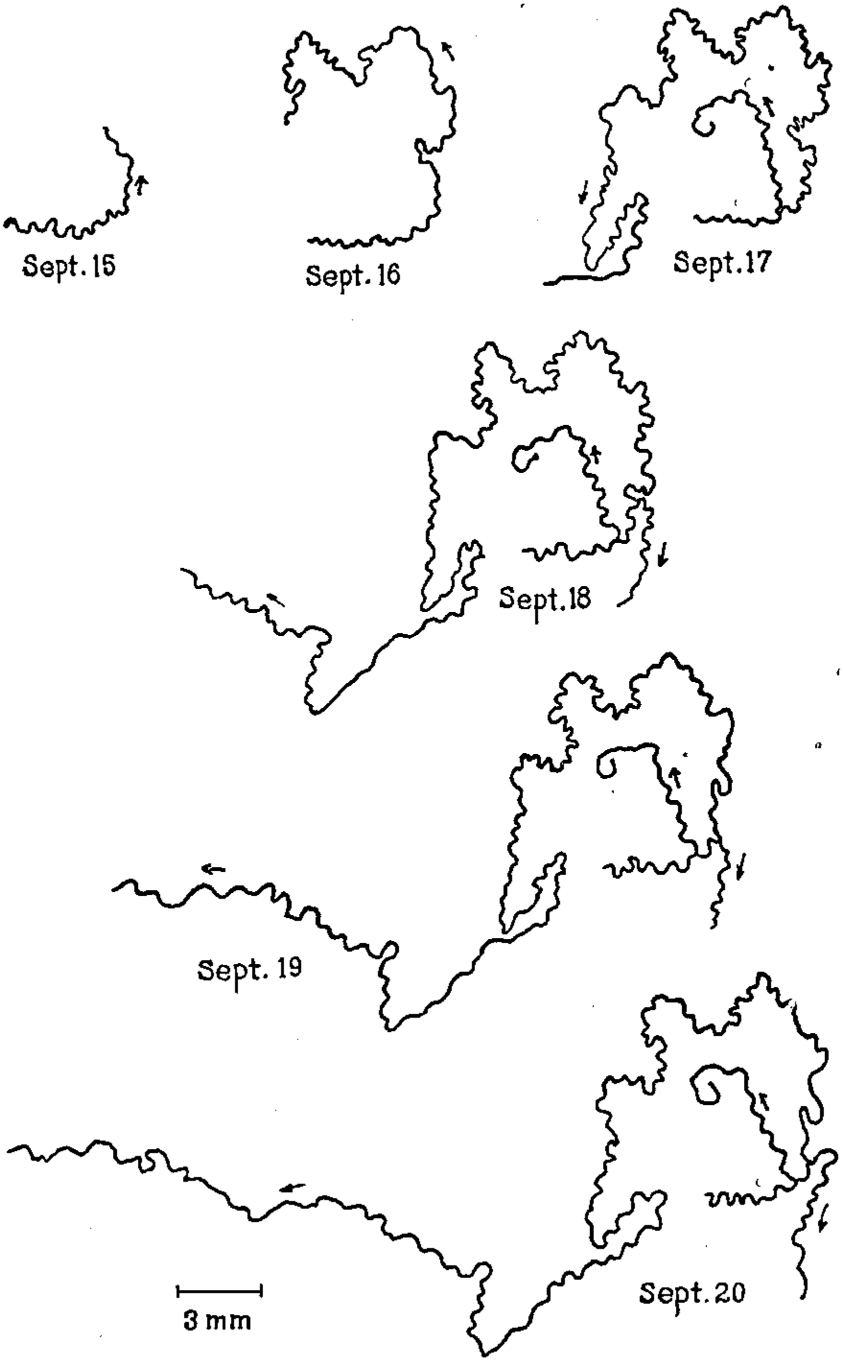

Fig. 1. Tracings of the trails made by the larvæ in the skin of volunteer 1, showing the progress of the migrating larvæ day by day. It is apparent that three larvæ were involved in the production of these linear eruptions.

the lesions easier or more convenient. As the writer was one of the two volunteers involved in these experimental infestations, it might be mentioned that the progress of the eruptions on his arm was subjected to an almost hourly subjective and objective observation.

Experiment 1.—C. M. A., the subject, was a male Filipino, 35 years old, without any previous hookworm infestation. Fæces consistently negative for hookworm eggs before the experiment. On September 4, 1931, fifty third-stage larvæ of *A. braziliense* were applied on the flexor surface of his arm. Typical lesions of creeping eruption were observed on the fifth day after the application of the larvæ. A week later, the eruptions developed to such an alarming proportion that it was decided to check their further advance by freezing them with ethyl chloride. The eruptions promptly disappeared after several applications of this remedy, but a few days afterwards, fresh linear eruptions suddenly flared up at the border of the original zone of infestation (fig. 5). Presumably these new eruptions were made by the few larvæ that escaped freezing, and which, after having lain dormant for a time, resumed their migratory activity. A daily tracing of these linear eruptions drawn to scale, was made for a period of six days in order to determine the rate of their progress (fig. 1). A few applications with ethyl chloride were enough to kill these left-overs so that by October 5, or a month after the application of the larvæ, the lesions had completely disappeared. It was apparent, however, that if left untreated, the eruptions would have spread and persisted for a considerable period. Stool still negative for hookworm eggs at this writing, October 30, 1931.

Experiment 2.—D. C. was a male Filipino, 19 years old, without any history of previous hookworm infestation. Fæces consistently negative for hookworm eggs before the experiment. Fifty third-stage larvæ of *A. braziliense* were applied on the skin of the flexor surface of his arm on September 4, 1931. The description of the course of the infestation in this case is closely parallel to that of case No. 1 described above, except for the fact that the volunteer afterwards developed a severe and extensive vesiculopustular dermatitis following bacterial infection due to scratching of the lesion. There was much swelling, redness, and pain of the parts involved, and for a time it was feared that he would develop an extensive cellulitis. Application of Dakin's solution alternated with freezing of the lesions with ethyl chloride for about a week resulted in recovery. Stool still negative for hookworm eggs at this writing, October 30, 1931.

DESCRIPTION OF THE ERUPTIONS

A few minutes after the application of the infective material, a smarting, pricking sensation is felt. Ten minutes after the infective material is washed off, small wheals ranging in size from the head of a pin to almost microscopic elevations appear. Most of the initial lesions are congregated at the edge of the infested zone. It seems that the larvæ are especially active in penetrating the skin at this area. A few hours later reddish papules replace these initial wheals, these papules being at first discreet but later on the erythematous areolæ surrounding them coalesce, thus forming a confluent erythemato-papular eruption. At the end of twenty-four hours minute vesicles appear, the zone of infestations now becoming the seat of an erythemato-papulo-vesicular type of dermatitis. The itching at this stage is almost intolerable. Only a keen desire on the part of the writer to produce natural and typical lesions has prevented him from scratching them, and this required the exercise of a considerable amount of self-restraint, as the eruptions are what one might call exquisitely itchy. On the fifth day, definite linear, serpiginous, tortuous advancing eruptions begin to appear. New linear eruptions are usually noted early in the morning as faint streaks of erythema which, as the day advances, become definitely raised tortuous lines which fade very gradually towards the advancing end. In another twenty-four hours or so, the serpiginous lesions become punctuated with small pearly looking vesicles which yield a watery fluid on being punctured. These vesicles do not become pustular unless secondarily infected with bacteria. In another twenty-four hours the tail end of the advancing linear eruptions dries up, leaving a dark, collapsing linear crust, which marks the trail of the migrating larva. Scratching of the lesions may lead to a serious vesiculo-pustular type of dermatitis, which if unchecked may even involve deeper tissues and start a dangerous cellulitis.

HISTOPATHOLOGY

Biopsies have been performed at various intervals during the course of the infestation experiments. Stained sections have been studied and the positions assumed by the migrating larvæ in the skin noted. Without exception, it has been noted that the larvæ are confined in the stratum mucosum. The larvæ are either tightly incarcerated or encapsulated by the proliferated epithelial cells (fig. 10), or lying loosely in tunnels (fig. 11), which have the stratum germinativum as the floor and the stratum granulosum or the stratum corneum as the roof. In the first instance, the epithelial cells surrounding the larva appear apparently normal, except those which are immediately in contact with the parasite, which show signs of cloudy swelling. It seems that the offending larva is well handled so that the toxic substance presumably excreted by the larva is well localized. On the other hand, the tissue reaction observed in the neighborhood of the larva, which occurs in tunnels, presents a different picture. Here œdema, marked cloudy swelling, and nuclear degeneration are noted. These are clear indications of certain toxic action exerted by the parasite on the surrounding tissue. That the larvæ of hookworms contain toxic substances is evidenced by the fact that "ground itch" has been produced experimentally by rubbing the skin with extracts of human hookworm larvæ (Chandler, 1930). Aside from round cell infiltration, regiments of eosinophiles are seen coming from under the corium in the direction of the offending parasite.

From the above study of the histopathology of the lesions of creeping eruption, it seems that the migrating larva is sometimes held in abeyance, or arrested in its migratory activity by the encapsulating power of the epithelial cells, and is therefore, unable to burrow its way to effect its advance. A struggle between the tissue cells and the parasite ensues, the body cells trying to imprison the parasite so as to localize its activity, while the larva throws out certain substances, or some kind of ferment with which it tries to dissolve or destroy the surrounding tissue. Sometimes the larva is successful and it breaks away from the clutches of the proliferated epithelial cells and starts burrowing tunnels in the stratum germinativum to effect migration. Sometimes the tissues are successful and the larva is kept in situ indefinitely until it dies and is calcified. This observation seems to find support in the fact that in the case of volunteer 1, new lines were noted to develop after periods of apparent inactivity on the part of the migrating larvæ. It seems quite likely that some of the larvæ in this case had been temporarily arrested in their activity by the tissue cells, and then succeeded in getting away.

DISCUSSION

The results of the above experiments tend to show that in so far as creeping eruption is concerned, the Filipino skin is just as susceptible to the invasion of the second stage larvæ of *A. braziliense* as that of the white population of the South Atlantic and the Gulf States of America, and that the explanation for the apparent absence of this skin disease in the Philippines must be sought in other directions. It is true that in the present work observations have been limited to the results of two experimental cases only. It may be asserted that no sweeping conclusions could be made with such limited data, but it is believed that such has been due, not to the difficulty in producing the lesions but rather to the lack of human volunteers. The ease with which typical lesions have been produced in the two cases involved in this work, gives ground for the belief that at least a large percentage of take could have been made if more volunteers were available. Indeed, it has been noted that the slightest carelessness in handling the infective material is enough to produce skin infestation with this larva. Considering the fact, however, that cats and dogs are popular pets in many Filipino homes, and that favorable climatic conditions for the proper development of the infective larval stages of *A. braziliense* are present throughout the year in this country, where sanitary facilities for the prevention of soil pollution are less adequate than in regions where this skin disease is frequently encountered, it is, indeed, rather surprising that not a single natural case of creeping eruption has so far been reported in this country.

A curious discrepancy which is of interest to mention, is that in Florida and its neighbor states where creeping eruption is frequently found, no human intestinal infestation of *A. braziliense* has so far been reported, whereas in the Philippines where human intestinal infestations with this species of hookworm have been rather frequently encountered, creeping eruption is apparently absent. This seems to suggest a duality of type of *A. braziliense.* It is possible that an Oriental type which has grown better adapted to the human host, and an occidental one, which is just in the first stage of adaptation, exist. In fact, from morphological grounds, Darling (1924) concludes that two definite types of this nematode exist; one which he calls the *ceylanicum* type, was first described as a distinct species under that name, and has been found in dogs, cats, and humans in India, Java, Malaya and the Philippines;

the other type occurs in South and Central America, and because it conforms better to the original description of the species described by de Faria, it is known as the *braziliense* type. It is quite possible that the cat and dog hookworms which cause creeping eruption in the Southern United States belong to the latter type. A study of the geographical distribution of the reported human infestations of *A. braziliense* seems to support this hypothesis.

The only report of human infestations of this worm from the western hemisphere is that of Gordon,(5) in which he recorded only 4 specimens of this worm out of 6,851 hookworms recovered from man in Amazonas, Brazil. On the other hand, human infestations of this worm have been reported many times in various countries of the Orient. In the Philippines Manalang (Chandler, 1929) found 11 per cent of 136 hospital cases infested with this worm, and found it to constitute about 1 per cent of 1,000 parasites obtained from Filipino inmates of a penal farm. Darling (1924) found 4 *A. braziliense* in 2,171 hookworms recovered from Malayans in the Malay Peninsula. In Burma, Jolly (Chandler, 1929) found 2.5 per cent of 7,629 hookworms recovered from man to be *A. braziliense.* Only 10 per cent of the Indian coolies who had been in Fiji for from two to ten years, harbored one or more hookworms of this species, with an average of 2.2 worms per infested man, whereas of 10 Indian coolies who had lived in Fiji for from 7 to 26 years, 31 per cent harbored *A. braziliense,* with an average of 4 worms per infested case (Chandler, 1929). Infection also occurs in India where Lane(9) found 20 specimens out of 2,625 hookworms as being of this species. In Rangoon Chandler(1) found 6 cases out of 7 autopsies harboring small numbers of ancylostomes (4 to 10) and no necators. Of the 6 infested individuals 5 harbored 2 or more *A. braziliense* and in one case 8 of the 10 worms were of this species. Chandler(1) states that "the presence of a heavy hookworm infestation is prima facie evidence of high susceptibility to hookworm infestation in general, which would probably be sufficient to allow an imperfectly adapted species to develop." This does not explain however, the relative absence of human infestations of this species of hookworm in the western hemisphere especially in the Southern United States where creeping eruption has been frequently reported, and where in certain areas the human hookworm burden of the population is just as heavy as in some Oriental countries.

In this connection it may be of interest to mention that a Japanese investigator after years of experimentation, seems to have succeeded in the establishing of human ascaris infestation in swine that had been kept on a vitamin-deficient diet for some time (Hall, 1930).(6) If it is true, as has been repeatedly alleged, that the rank and file of the Oriental population live on a vitamin-deficient diet, the apparent higher intestinal infestation among Orientals with *A. braziliense* might have been due to the existence of a better adapted type of this species of hookworm, which has been evolved in the course of time in this part of the world. This might also explain the frequent occurrence of creeping eruption caused by the larvæ of this worm in the southern United States and the apparent absence of the same in the Philippines and other Oriental countries. If the above conjecture is correct, it might be assumed that a great proportion of the larvæ, of the type better adapted to the general population of Oriental countries, promptly enter the circulation after penetrating the skin and develop into maturity in the intestine, as would the larvæ of the "normal" human hookworms, instead of staying in the skin and performing horizontal migrations that give rise to the typical lesions of creeping eruption. It is true that typical lesions of this skin disease have been produced in the two Filipino volunteers involved in this work, but they could hardly be classed among the vitamin-deficient portion of our population, as one is a physician and the other a well-nourished laboratory technician. It would be interesting to learn the results of similar infestation experiments in a larger series, performed on persons picked from the general population in the Philippines.

It seems quite probable, therefore, that other factors still unknown have a hand in the causation of this discrepancy in the geographical distribution of both the intestinal human infestations of *A. braziliense* and creeping eruption. This offers a fascinating field for students of host-parasite relations.

SUMMARY

Creeping eruption of the type found by Dove and White(3) to be due to the penetration of the skin by the third stage larvæ of *Ancylostoma braziliense* in the South Atlantic and the Gulf States of America, has been successfully produced experimentally on two Filipino volunteers with the infective larvæ of this species of hookworm, which heavily parasitizes cats, and to a certain extent dogs and humans in the Philippines. The apparent absence, therefore, of natural cases of creeping erup-

tion among the Filipinos cannot be explained on the ground of a racial resistance or immunity of the latter against the invasion of the skin by the larvæ of *A. braziliense.* The explanation for this must be sought in other directions.

The apparent discrepancy in the geographical distribution of creeping eruption and human intestinal infestations of *A. braziliense* seems to support Darling's contention that two distinct types of this species of hookworm exist; namely, an Oriental or a better-adapted type, and an Occidental type, which is just beginning to adapt itself to the human host. That a difference in the vitamin content of the diet of the two groups of people involved might have had something to do in the evolution of these two presumably distinct types of *A. braziliense,* seems probable.

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ILLUSTRATIONS

PLATE 1

FIG. 1. Showing the initial lesions of creeping eruption photographed ten minutes after the application of the infective third-stage larvæ of *Ancylostoma braziliense* on the flexor surface of the arm of volunteer 1.

2. The infested zone on the arm of volunteer 1, twenty-four hours after the application of larvæ.

PLATE 2

FIG. 1. The same infested area forty-eight hours after the application of larvæ.

2. The same arm eighteen days after infestation, showing typical lesions of creeping eruption. X marks the original zone of infestation.

PLATE 3

FIG. 1. Creeping eruption on the arm of volunteer 2, photographed five days after the application of the third-stage larvæ.

2. The same arm of volunteer 2 on the seventh day after the application of the infective larvæ.

PLATE 4

FIG. 1. The same arm of volunteer 2 on the fourteenth day after infestation.

2. The arm of volunteer 2 developed a severe dermatitis following bacterial infection due to scratching. Photographed when the case was fairly well under control with Dakin's solution. Note that the characteristic linear eruptions have been effaced.

PLATE 5

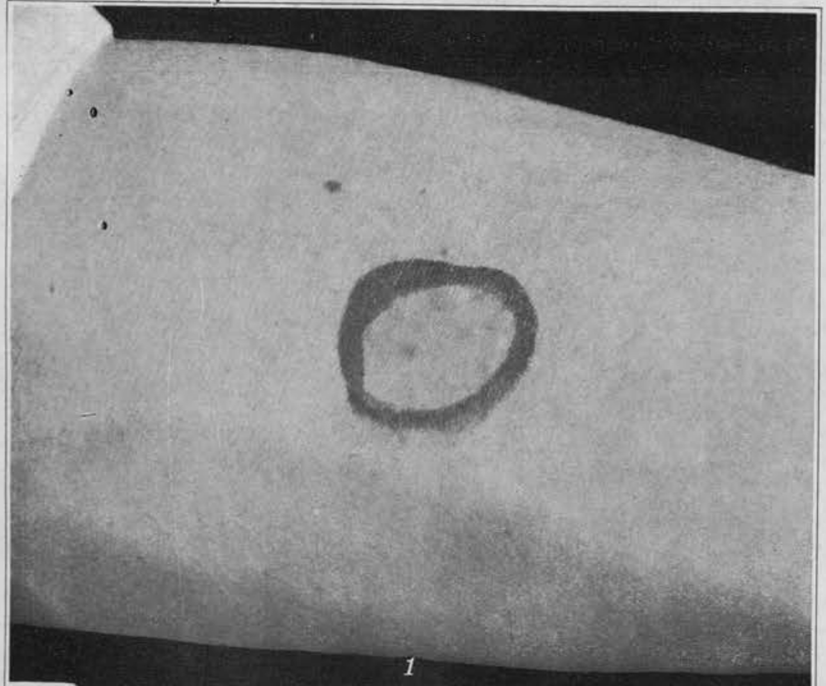
FIG. 1. The infective third-stage larva of *Ancylostoma braziliense* from an 8-day fæcal culture from a cat.

2. Microphotograph of a section of the skin showing segments of the burrowing larvæ which are confined in the stratum germinativum. Note the flattening of the epithelial cells around the larva.

3. Cross section of a tunnel formed by the burrowing larva. Note the disintegrating larva just under the stratum corneum, the swollen epithelial cells with nuclear degeneration in the neighborhood of the parasite, and the stream of eosinophilic infiltration that comes from beneath the stratum Malphigi in the direction of the offending parasite.

TEXT FIGURE

FIG. 1. Tracings of the trails made by the larvæ in the skin of volunteer 1, showing the progress of the migrating larvæ day by day. It is apparent that three larvæ were involved in the production of these linear eruptions.



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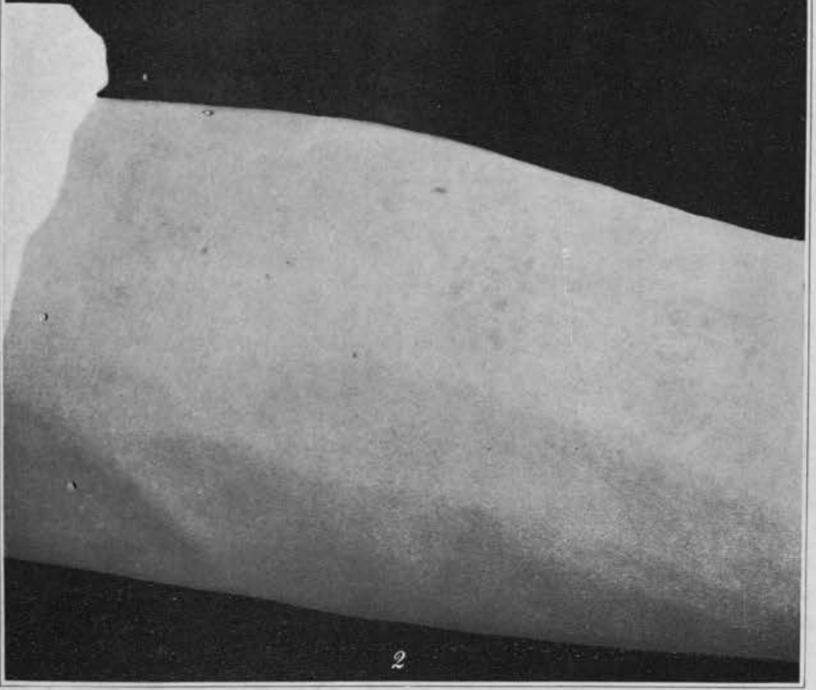

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PLATE 1.

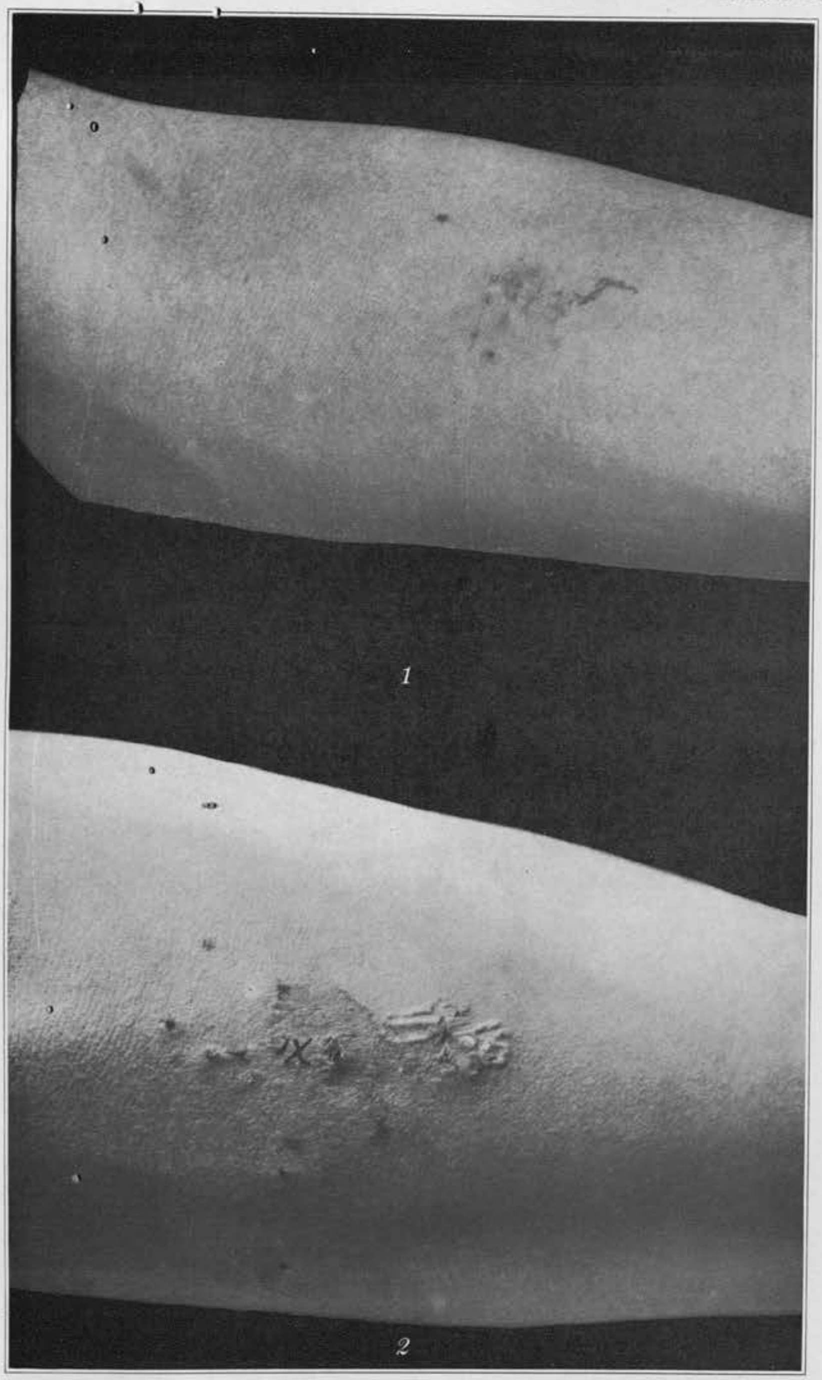
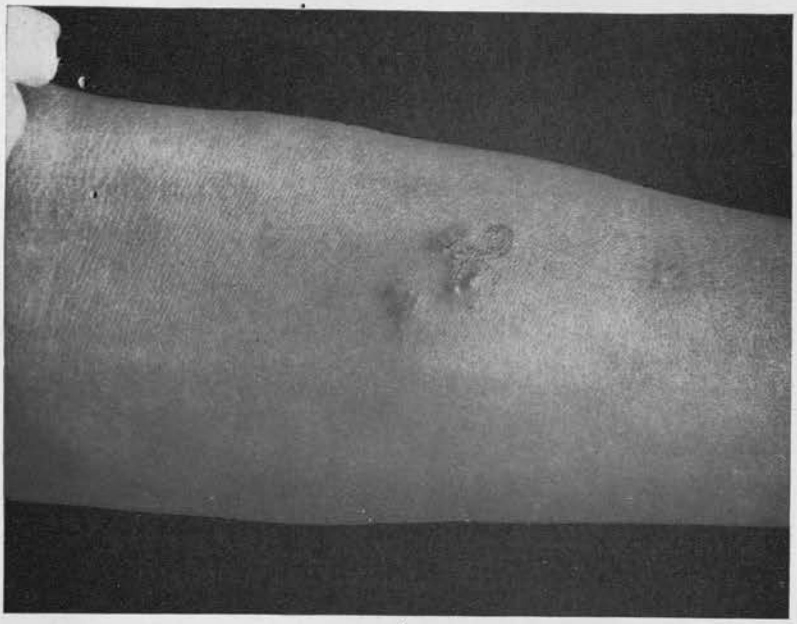


PLATE 2.



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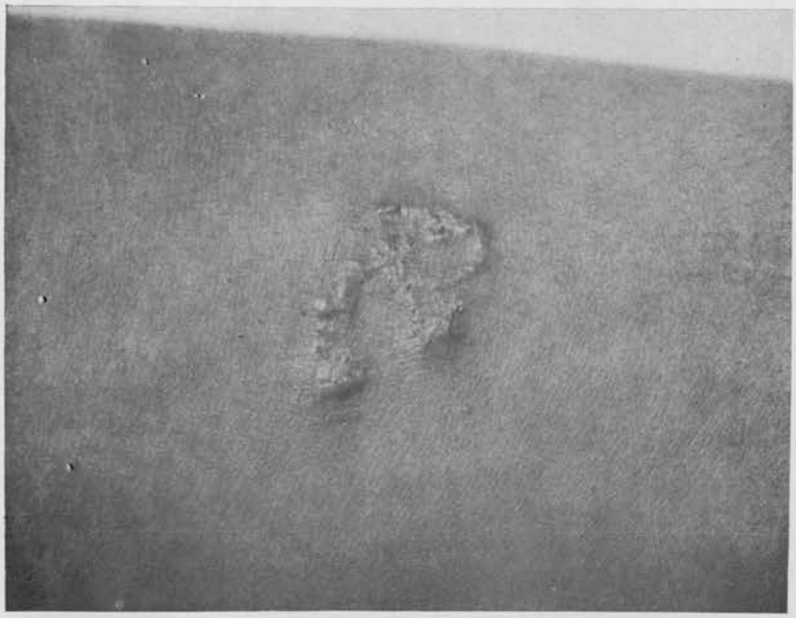

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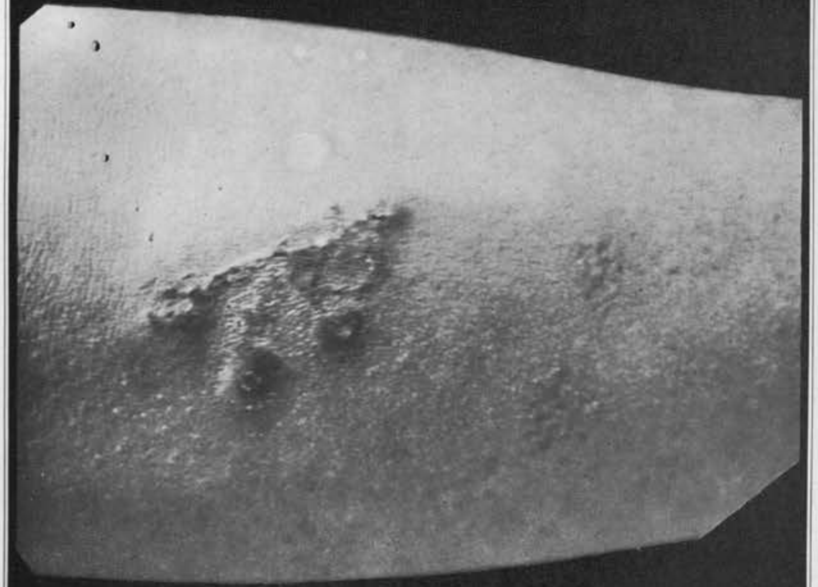
PLATE 3.



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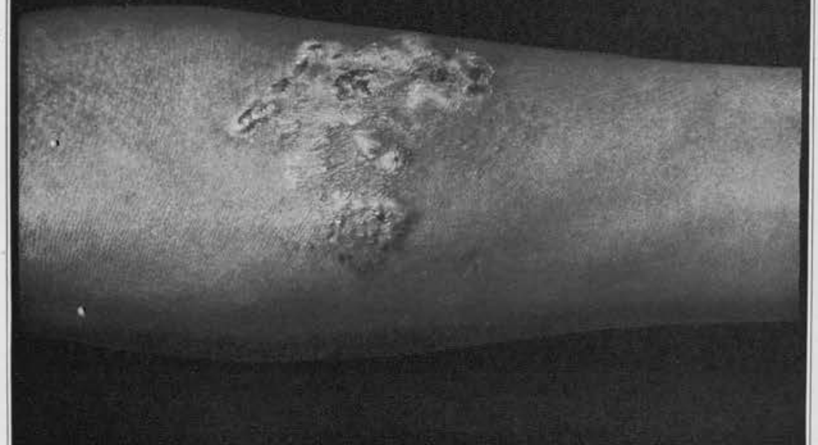

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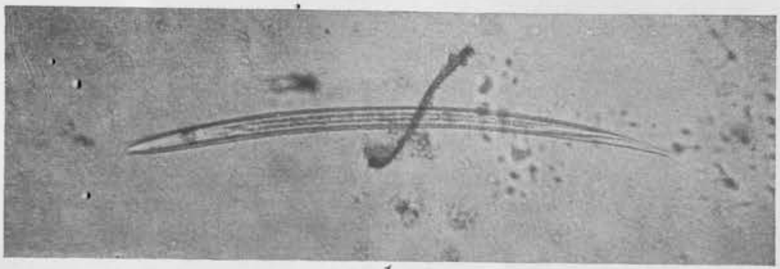
PLATE 4.



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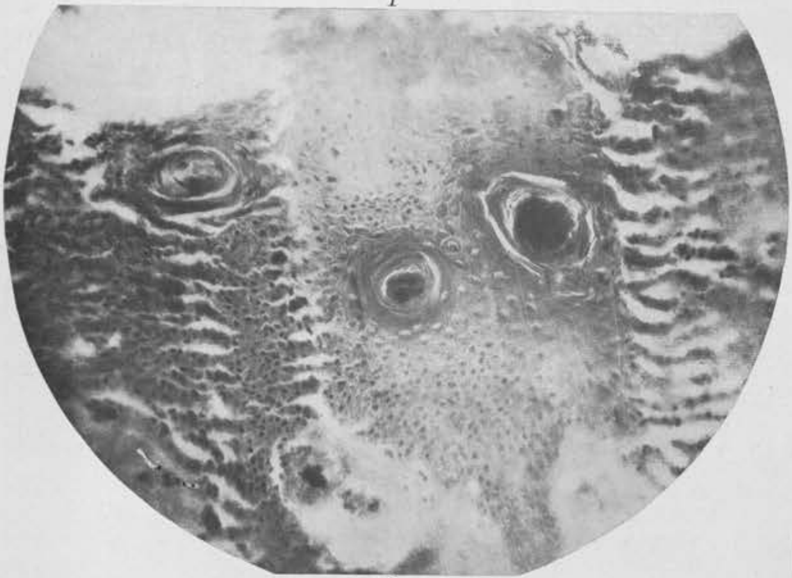

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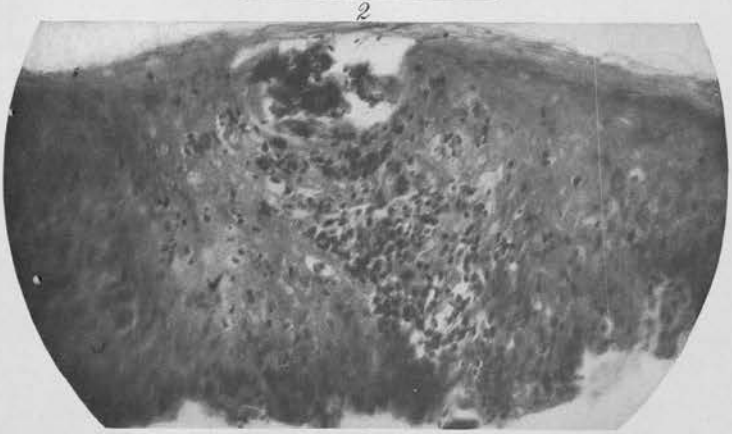

3

PLATE 5.

STUDY ON A PHILIPPINE STRAIN OF LEPTOSPIRA ICTEROHÆMORRHAGIÆ [1]

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Of the Division of Biology and Serum Laboratory
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ONE TEXT FIGURE

INTRODUCTION

The absence of infectious jaundice (Weil's disease) from the Philippine Islands has frequently been remarked upon. McKinley [3] remarks—

> In so far as we have been able to learn, epidemics of so-called infectious jaundice have never occurred in the Philippines. Indeed the writer has been unable to find any record in the various hospitals in Manila or in the Naval Hospital at Cañacao of a single case of infectious jaundice associated with the presence of the *Leptospira icterohæmorrhagiæ.* This is a curious observation in view of the widespread prevalence of this disease in Japan and Formosa and the extensive shipping communications which exist between these countries and the Philippines.
>
> * * * * * * *
>
> In rat series 192 to 197 the pooled blood contained spirochetes and guinea pigs receiving 5 cubic centimeters of blood intraperitoneally from this series of rats developed typical jaundice and cultures of *Leptospira icterohæmorrhagiæ* were obtained from the kidneys of the dead guinea pigs. Healthy guinea pigs inoculated with kidney emulsion of these animals developed jaundice. The cultures from the original guinea pigs unfortunately became contaminated and were lost. We were never able to use these cultures for experimental infections and did not attempt to isolate others since we were interested solely in determining the presence of the parasite. Subsequent examination of over 50 rats has never resulted in another positive result. None of the kidney sections have shown spirochetes. From the morphological, pathological and cultural characteristics of these spirochetes we feel certain that they are *Leptospira icterohæmorrhagiæ.*

In addition to the evidence produced by McKinley which shows that infection with *Leptospira icterohæmorrhagiæ* exists among

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[1] Received for publication November, 1931.

[2] Lieutenant Colonel, Imperial Japanese Army.

[3] Proc. Soc. Exp. Biol. & Med. 26 (1928–29) 26–28.

rats in Manila, we are presenting herewith further proof of the presence in the Philippines of Weil's disease as a contribution to the nosography of this disease. The study of the Manila strain of *Leptospira icterohæmorrhagiæ*, as presented in this publication, leaves no doubt of the identity of the parasite. It shows the possibility of the disease being contracted by man and the biologic peculiarity of the Manila strain may explain the paucity of the disease among humans as well as among rats in Manila.

THE SOURCE OF MATERIAL

A Japanese, residing continuously in Manila for three years, suddenly became ill. According to the relatives of the patient the illness commenced with vomiting and fever. These symptoms soon were joined by jaundice and anuria, according to the statement of the patient's relatives. The patient was removed to a local hospital where he died. Due to the advanced stage of the disease only urine was secured for laboratory examination, and investigation was carried out by laboratory experiments in search of *Leptospira icterohæmorrhagiæ.*

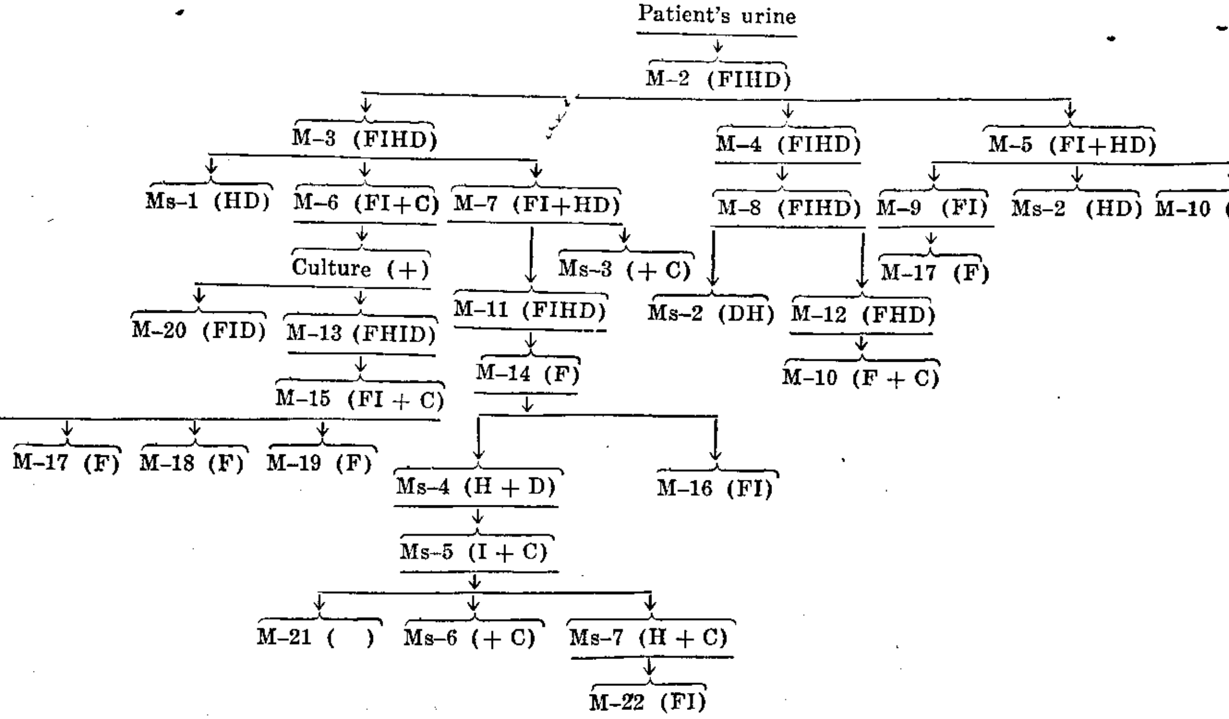
PROCEDURE OF EXPERIMENTAL INVESTIGATION

About 8 cubic centimeters of the patient's urine was centrifuged and the resulting sediment together with 2 cubic centimeters of the supernatant fluid was injected subcutaneously into a guinea pig weighing 130 grams. Microscopic dark-field examination of the urine sediment failed to reveal spirochætes. The inoculated guinea pig developed considerable swelling at the point of inoculation. The swelling gradually localized and an abscess formed at the place of inoculation. The pus obtained from the abscess was examined under the dark-field microscope, but no spirochætes were found. There was distinct jaundice discolorization noticeable around the abscess and a slight general icterus.

At this time, thirteen days after inoculation, about 0.2 cubic centimeter of this animal's blood was withdrawn and injected intraperitoneally into a normal guinea pig. The next day; that is, the fourteenth day after inoculation the first guinea pig that was inoculated with the patient's urine died. During the last few days of sickness, the animal showed moderate fever. Autopsy performed on the inoculated animal showed typical findings of experimental Weil's disease. Further passages through guinea pigs are given in Table 1.

TABLE 1.—*Showing the successive passages of Manila strain of Leptospira icterohæmorrhagiæ.*

[M, guinea pig; Ms, white mouse; D, died; C, carrier; F, fever; I, icterus; H, hæmorrhages; +, leptospiræ found by darkfield.]



ANIMAL INOCULATION

Subsequent inoculations of experimental animals were performed by intraperitoneal injection. The experimental animals used were guinea pigs and white mice.

CULTIVATION OF THE PHILIPPINE STRAIN OF LEPTOSPIRA ICTEROHÆMORRHAGIÆ

The culture medium employed in cultivation was Ohba's medium, consisting of tap water that contained 0.1 per cent calcium chloride.

The inoculum was blood obtained from living guinea pigs showing symptoms of the infection. The inoculated culture media were incubated at a temperature of 36.5° C. under aërobic conditions.

The identity of the spirochætes obtained in the culture with *Leptospira icterohæmorrhagiæ* was proven by dark-field, stained smears, and Levaditi sections. Both guinea pigs inoculated with the culture died with typical symptoms and autopsy findings.

MORPHOLOGY AND CULTURAL CHARACTERISTICS

In its morphology, as observed in material obtained from inoculated animals and from cultures, the parasite was identical with those strains observed by the author on many occasions in Japan.

The morphology of the parasites in question was studied in direct slides obtained from organs at autopsy, in blood and urine during life. Aside from dark-field examination, smears were prepared from the above-mentioned materials and stained by Fontana's method, carbol fuchsin, carbol gentian violet, and Giemsa stain. Sections were stained by the Levaditi method.

CLINICAL PICTURE OF EXPERIMENTAL INFECTION WITH MANILA STRAIN OF LEPTOSPIRA ICTEROHÆMORRHAGIÆ IN GUINEA PIGS

The clinically noticeable signs of experimental Weil's disease are fever and jaundice. Mortality is high among inoculated guinea pigs.

A few days after the inoculation, the body temperature rises, and reaches the highest point from the fifth to the eighth day after inoculation. This high rise of temperature is followed by a sudden drop. In the most acute course of experimental infection the curve plunges from the highest peak down to subnormal temperature, and death occurs within one or two days after the peak of the temperature curve has been reached. (See fig. 1, temperature curve of W–M–3, 4, 7, 11, 13.) The type of fever is intermittent, as is well revealed in those animals that survived. In such cases the repeated elevations are plainly discernible, each subsequent rise being less than the preceding one. (See fig. 1, temperature curve of W–M–6.)

AUTOPSY FINDINGS IN GUINEA PIGS

The post-mortem findings in those animals that died of infection by *Leptospira icterohæmorrhagiæ*, isolated in Manila, varied somewhat as to intensity but they were typical in every case.

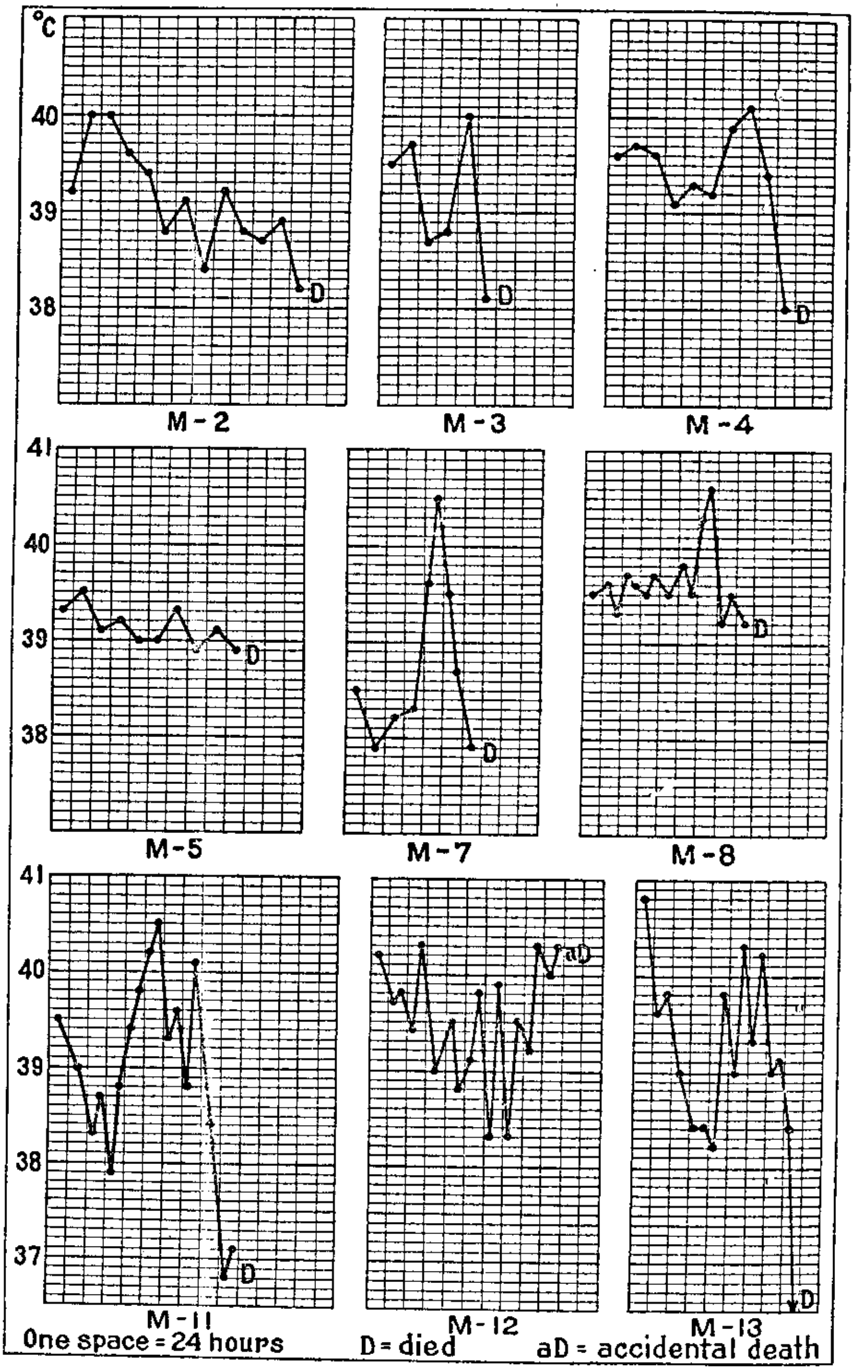
The characteristic jaundice plainly discernible before and after death upon external examination was in every case much more striking in the subcutaneous tissue. The axillary and inguinal lymph glands were enlarged and hæmorrhagic. Petechial hæmorrhages scattered throughout the subcutaneous tissue. Internal organs hyperæmic, showed the typical yellowish tinge. The lungs exhibited scattered hæmorrhages more regularly and to a greater extent than the other internal organs.

Microscopic examination confirmed the autopsy findings as to the hæmorrhages. Parenchymatous cells were found degenerated. Sections prepared from pieces of liver that were stained by Levaditi's method presented fairly numerous spirochætes. These were scattered, isolated, throughout the parenchyma of the liver and occasionally in groups, and clusters of the leptospiræ were encountered in the interstitial tissue.

CARRIERS IN EXPERIMENTAL ANIMALS INOCULATED WITH MANILA STRAIN OF LEPTOSPIRA ICTEROHÆMORRHAGIÆ

In the course of inoculations from animal to animal, it was observed that occasionally guinea pigs developed fever and even icterus, but recovered. The leptospiræ could not be demonstrated in the blood of these animals by microscope. However, a certain time after recovery numerous leptospiræ were found in the urine of the animals. The elimination through urine of leptospiræ in these animals was intermittent. Samples of urine contained at times numerous leptospiræ, while it was impossible to demonstrate them in the urine of the same animals the next day.

It appears significant that none of the animals inoculated with the urine obtained from carriers and which contained numerous leptospiræ died of the infection. Some, however, presented typical fever (M–19).


°C
40
39
38
D
M-2
D
M-3
D
M-4
41
40
39
38
D
M-5
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M-7
D
M-8
41
40
39
38
37
D
M-11
aD
M-12
D
M-13
One space = 24 hours
D = died
aD = accidental death

FIG. 1.

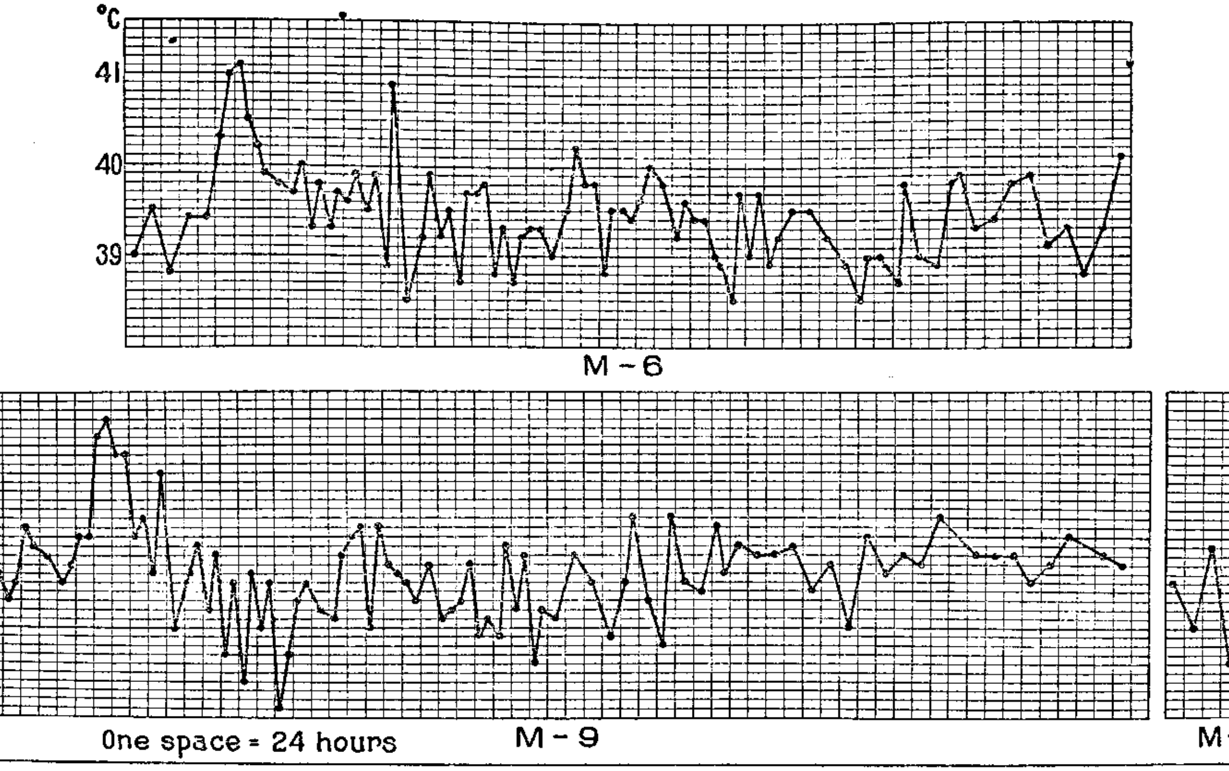

FIG. 1a.

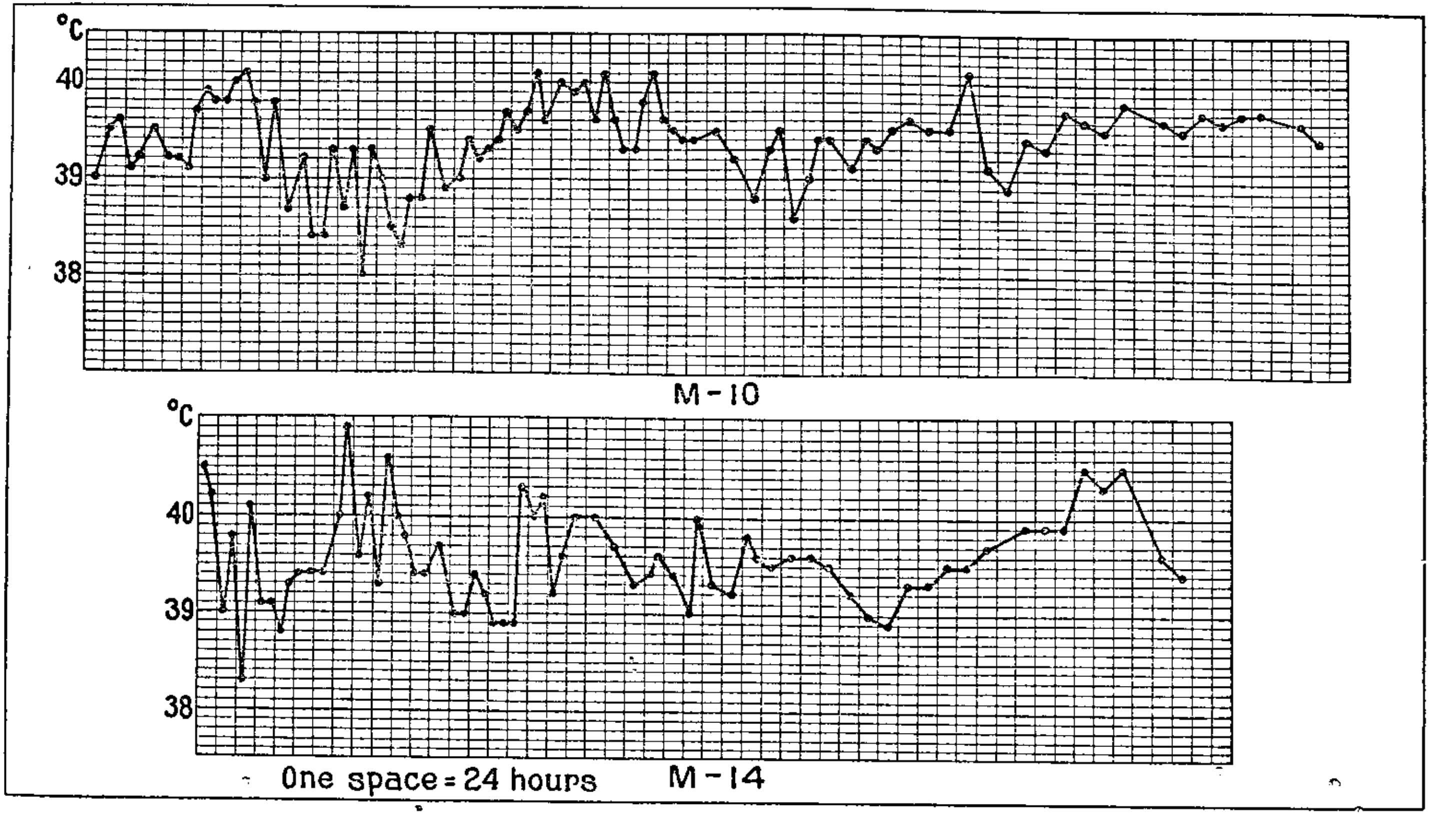

FIG. 1b

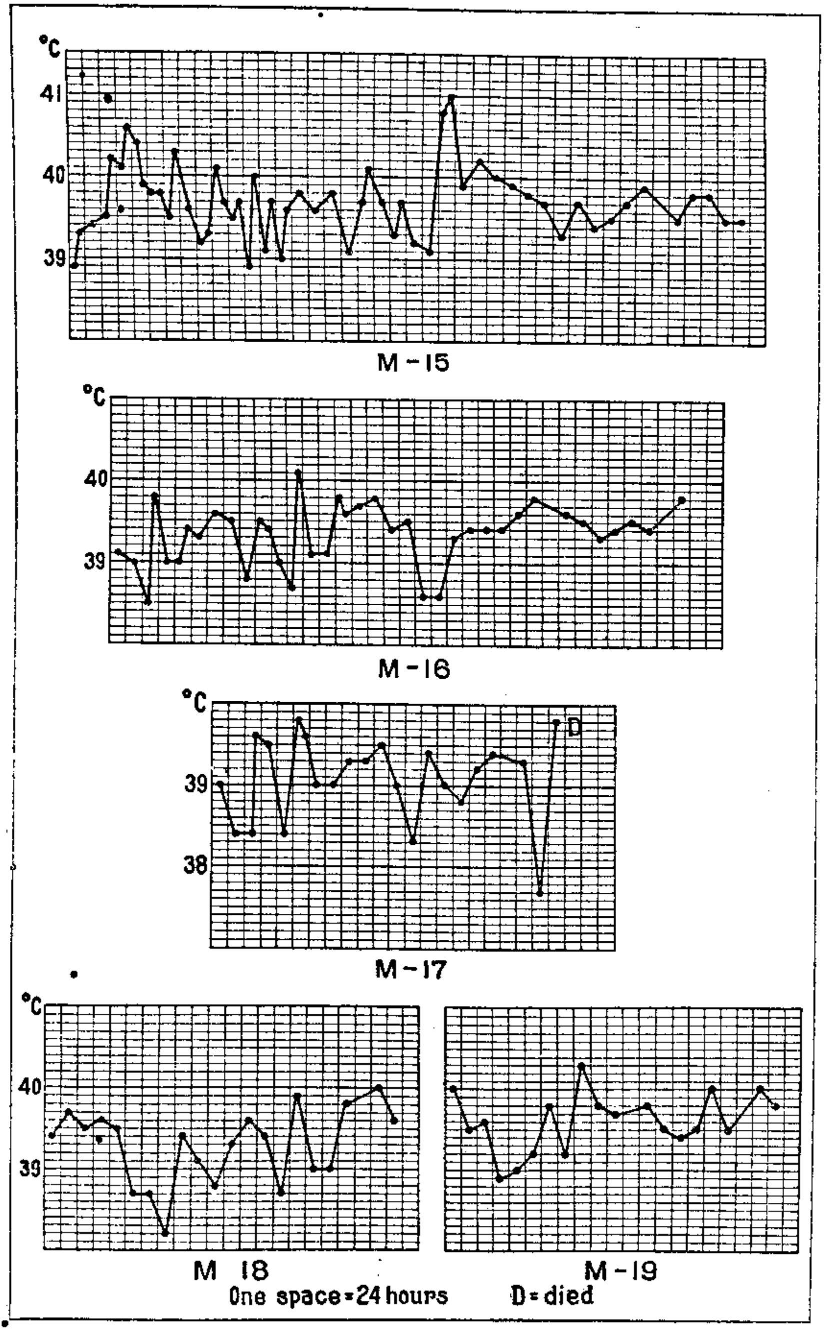
°C
41
40
39
M-15
°C
40
39
M-16
°C
D
39
38
M-17
°C
40
39
M 18
M-19
One space=24 hours
D=died

FIG. 1c.

The mortality among white mice inoculated with the Manila strain of *Leptospira icterohæmorrhagiæ* was even lower than that among guinea pigs. The carrier state, however, was frequently encountered in these animals. The carrier state could be transmitted from mouse to mouse while the donor as well as the recipient remained alive.

SUMMARY

From the urine of a fatal case of infectious jaundice, in a long time resident of Manila, morphologically and biologically typical *Letospira icterohæmorrhagiæ* was isolated. Typical symptoms; that is, fever, hæmorrhages, and icterus, were produced in guinea pigs by passage inoculation and leptospiræ were demonstrated in these animals by dark-field, stained preparations, and Levaditi sections. The virulence to experimental animals (guinea pigs and white mice) was found relatively low in comparison with the virulence of the Japanese strains studied. The attempt to increase the virulence by successive passages through susceptible animals (guinea pigs and white mice) had, as a consequence, diminished rather than increased virulence. While more than half of the passage animals died an acute death with typical clinical and post-mortem findings, some of them recovered, showing only mild or no clinical symptoms. Of those that recovered, a certain number became carriers, discharging in their urine large numbers of leptospiræ, which when transferred to normal animals failed to produce death, but induced symptoms and carrier state.

CONCLUSION

The existence of Weil's disease in the Philippine Islands, indicated by previous findings among rats by McKinley, is confirmed by demonstrating *Leptospira icterohæmorrhagiæ* in the urine of a fatal human case of infectious jaundice.

The strikingly low virulence of the Manila strain, particularly as regards animal carriers, is offered, for the present, as an explanation of the paucity of Weil's disease in Manila among rats as well as humans.

ILLUSTRATION

Text fig. 1. Chart showing temperature curves of guinea pigs inoculated with a Philippine strain of *Leptospira icterohæmorrhagiæ*.

OBSERVATIONS ON THE POSSIBLE TRANSMISSION OF SURRA BY THE LAND LEECH HÆMADIPSA ZEYLANICA

By MARCOS A. TUBANGUI
Of the Division of Biology and Serum Laboratory
Bureau of Science, Manila

ONE PLATE

INTRODUCTION

One of the biological differences that may be said to exist between the trypanosomes of fishes and other aquatic cold-blooded vertebrates and the trypanosomes of mammals and other warm-blooded vertebrates is their mode of transmission. So far as known, the trypanosomes of the former group of animals are conveyed by leeches while those of the latter group are usually transmitted by blood-sucking insects. These modes of propagation are readily understood if it is recalled that the transmission of specific infections by intermediaries is only possible if the animals harboring the infections are easily accessible to the transmitting agents. This being the case, it could hardly be suspected that blood-sucking insects would serve as appropriate transmitters for the trypanosomes of aquatic cold-blooded vertebrates. But it so happens in the case of leeches that they also attack warm-blooded vertebrates, especially mammals, and it is evidently for this reason that they have also been incriminated in the transmission of mammalian trypanosomes.

During an outbreak of horse trypanosomiasis in Annam, Vassal (1906) carried out some experiments in order to determine the mode of propagation of the disease. He used leeches, ticks, fleas, and several species of blood-sucking flies. His results were negative, but he made an interesting observation that the trypanosome responsible for the disease was unable to live long in the digestive tract of the leeches (species not mentioned). He tested the blood ingested by the annelids from infected animals by injecting it into rats and found that it was infective immediately after a meal of blood but not four hours later.

In the Philippines Boynton (1913), while engaged in looking for an invertebrate reservoir host of the virus of rinderpest, inquired into the possible transmission of surra by the carabao leech, *Hirudinaria manillensis*. He fed leeches partially on guinea pigs heavily infected with surra trypanosomes and after lapses of certain lengths of time (7 minutes to 2 days) allowed them to bite healthy animals. He also triturated one leech in physiological salt solution two days after it sucked infected blood and injected it into a normal guinea pig. The results of the experiments were all negative and it was concluded that the carabao leech cannot hold the trypanosome of surra alive for any length of time and that it cannot transfer the disease by biting.

In a recent publication Seegert (1930) has incriminated leeches (species not mentioned) in the spread of Mal de Caderas in Brazil. He cites the observations of Brazilian farmers to the effect that in outbreaks of the disease the removal of infected herds to high places where there are new sources of water supply serves to prevent the propagation of the infection. Similar results are said to be obtained in spite of the presence of large numbers of *Tabanus* flies, which are the suspected carriers of the disease, if sick horses are separated from healthy ones by a simple wire fence. According to Seegert, the incidence of Mal de Caderas in Brazil is low during the months immediately preceding the rainy season when *Tabanus* flies are very numerous; on the contrary it is high when these flies are scarce, coinciding with the prevalence of leeches, which in tropical countries are said to hibernate during the dry season and begin to come out shortly before and after the rainy season. The people are said to see to it that their animals avoid certain known water holes, due to a traditional belief that horses will contract the disease if allowed to drink at these places. Seegert examined leeches from one of these water holes where a sick animal had been found. He encountered trypanosomes that he believed to have something to do with Mal de Caderas but which Doctor Cesar Pinto, of the Instituto Oswaldo Cruz, whose opinion was sought, considered as developmental stages of a frog parasite. Finally Seegert cites Professor Doctor von Bassewitz, of Porte Alegre, who used leeches in treating his riding horse (what the treatment was for is not stated), which developed Mal de Caderas as a result of the treatment. The case was reported at a meeting of naturalists in Hamburg in 1928.

In view of this uncertainty of opinion on the rôle of leeches in the transmission of mammalian trypanosomes and because it was felt that the question has not yet been thoroughly studied, judging from the scanty literature available, it was decided to undertake the present reported work.

MATERIALS AND METHODS

The trypanosome studied is a strain of *Trypanosoma evansi* which was originally obtained from a surra horse about a year ago and has since been maintained in the laboratory by direct passage to guinea pigs.

Two species of leeches were utilized in the transmission experiments: the water leech, *Hirudinaria manillensis* (Lesson) (=*Hirudo boyntoni,* Wharton, 1913), commonly known as the rice-field or carabao leech (Plate 1, fig. 1) and the land leech, *Hæmadipsa zeylanica* (Plate 1, fig. 2). The former, as one of its names imply, is commonly met with in rice fields and also in swamps, ponds, and sluggish streams in the costal provinces of India, Ceylon, China, and the Malay Islands including the Philippines. It is often employed as a medicinal leech in many of these countries. Among the domesticated animals, carabaos are its most frequent victims and, as already mentioned, it is the form used by Boynton in his experiments on rinderpest and surra.

The land leech, *Hæmadipsa zeylanica,* including its several varieties, is found in humid places, especially in the lower ranges of the hill-countries of southeastern Asia, the Malay Archipelago including the Philippines, and other Oriental countries. It is a tormenting pest to human beings and animals travelling through mountain forests in these regions. Tennent (1861), Wallace (1902), and other naturalists who visited these countries all wrote accounts of their experiences with this leech. Tennent says that "Horses are driven wild by them (land leeches); they stamp the ground in fury to shake them from their fetlocks, to which they hang in bloody tassels."

Before starting on the transmission experiments, it was considered necessary to perform preliminary viability tests in order to ascertain how long the surra parasite is able to survive in the bodies of the leeches after it is sucked by the latter with the blood of infected animals. If it is determined that the trypanosome cannot remain alive in the annelids for a reasonable length of time, then there is very little possibility, if any, of the disease being carried by the leeches. The method employed consisted in allowing the annelids to feed on heavily infected laboratory animals until they became engorged with blood. Then from time to time samples of blood were drawn from their digestive tracts by means of fine sterilized needles and syringes and injected subcutaneously into white mice. In some cases where it was difficult to obtain blood with a syringe, the leeches were cut into small pieces and triturated with sterile salt solution and the fluid portion of the mixture injected into mice and guinea pigs.

The transmission experiments were carried out in the manner that similar experiments on surra have been performed with the use of blood-sucking insects; that is, by means of interrupted feedings. One or more leeches of the same species were allowed to bite a heavily infected laboratory animal for a few minutes. They were then removed to clean containers and after varying intervals of time (1 to 105 minutes) they were placed on a healthy host. In some cases they were kept alive for much longer periods, up to one and a half months, after which they were examined carefully for living trypanosomes or developmental stages of these organisms. They were then cut into small pieces and triturated with sterile physiological salt solution and were either fed or injected (the supernate) to mice and guinea pigs.

Usually it was not found difficult to make the leeches continue their blood meal when removed from one animal to another. When newly captured and hungry, they attached themselves readily to any portion of the body surface of a mouse, rat, or guinea pig, but preferably to the inner surfaces of the hind legs, the soles of the feet, or around the mammary glands where the hair is less dense or absent. The only precaution taken was not to allow them to suck too long (not more than 4 minutes from the time they began to suck) from the first animal, otherwise it was difficult or even impossible to induce them to take a second meal of blood. In the case of the land leech, however, after it had been kept in the laboratory for a few days, it usually became less active and less disposed to bite. It was induced to suck by shaving the skin or by applying it on wounded surfaces where other leeches had previously bitten.

RESULTS OF EXPERIMENTS

For convenience, the results of the various experiments have been condensed and incorporated in Tables 1 to 5.

Table 1.—*Length of time Trypanosoma evansi remains infective in the digestive tract of Hirudinaria manillensis.*

Interval after biting infected host.	Blood from leech injected to mouse No.—	Date of injection (1931).	Fate of animal receiving injection.
H. m.			
0 10	1	May 26	Positive June 1; died June 4.
1 20	4	June 1	Positive June 6; died June 8.
2 30	5	do	Negative; kept under observation for 25 days.[a]
3 28	6	do	Do.
72 0	3	do	Do.

[a] In this and in the rest of the experiments, in which negative results were obtained, the susceptibility of the different animals was subsequently proven by inoculating them with viable trypanosomes.

Length of time Trypanosoma evansi survived in the digestive tract of Hirudinaria manillensis.—As shown in Table 1, the length of time that the surra trypanosome was able to live in the digestive tract of the carabao leech was quite brief. Blood samples taken from leeches up to one hour and twenty minutes after they were allowed to bite heavily infected animals were infective when injected to mice, but not after two and a half hours. Microscopic examinations of the gut contents, when they were no longer infective, revealed the presence of numerous dead trypanosomes. They appeared stiff and without visible undulating membranes.

Table 2.—*Length of time Trypanosoma evansi remains infective in the digestive tract of Hæmadipsa zeylanica.*

Interval after biting infected host.	Blood from leech injected to—	Date of injection (1931).	Fate of animal receiving injection.
H. m.			
1 0	Mouse 8	June 11	Positive June 16; died June 19.
2 0	Mouse 9	do	Positive June 16; died June 18.
3 0	Mouse 13	July 3	Positive July 8; died July 13.
3 20	Mouse 10	July 11	Positive July 16; died July 19.
4 15	Mouse 11 and rat 1	July 17	Mouse positive July 25, died July 30; rat positive July 27, died August 2.
5 45	Rats 2 and 3	do	Both negative; kept under observation for 50 days.
23 20	Mice 12 and 18	July 21	Both negative; kept under observation for 46 days.

Length of time Trypanosoma evansi survived in the digestive tract of Hæmadipsa zeylanica.—As shown in Table 2, the period of time that the surra trypanosome was able to remain alive in the digestive tract of the land leech was much longer than in the carabao leech. Blood samples taken up to four hours and fifteen minutes after the leeches were allowed to suck from infected animals were infective when injected to mice, but they were not infective after five hours and forty-five minutes. Microscopic examinations of the gut contents when they were no longer infective, revealed the presence of numerous disintegrating trypanosomes and, in some cases, motile ones or what appeared to be their developmental forms. These latter, however, as will be shown later, have nothing to do with the surra parasite.

TABLE 3.—*Direct transmission experiments with Hirudinaria manillensis.*

Interval between bites from infected to healthy animal.	Number of leeches applied.	Healthy animal exposed to leeches.	Fate of animal exposed.
Min.			
1	2	Guinea pig 1	Negative; kept under observation for 29 days.
3	1	Rat 2	Do.
10	2	Guinea pig 2	Negative; kept under observation for 32 days.
60	3	Guinea pig 3	Do.
75	2	Guinea pig 5	Negative; kept under observation for 35 days.
30	1	Mouse 14	Negative; kept under observation for 28 days.
3	3	Guinea pig 6	Negative; kept under observation for 35 days.
5	1	Rat 3	Negative; kept under observation for 25 days.

Transmission experiments with Hirudinaria manillensis.—As shown in Table 3, all attempts to transmit surra directly through the bites of one or more carabao leeches resulted negatively. The intervals between their bites from infected to healthy animals were from one to seventy-five minutes and were, therefore, within the length of time during which the surra trypanosome has been proven to remain alive in the digestive tract of *Hirudinaria manillensis.*

Some trypanosomes, such as *Trypanosoma lewisi, T. gambiense,* and others, are said to lose their virulence temporarily a few hours after they are taken up by their respective intermediate hosts and do not become infective again until after they have passed through definite cycles of development. To determine if *T. evansi* undergoes cyclic changes within the body of *Hirudinaria manillensis,* leeches that had sucked infected blood were kept alive for twenty-nine to forty-four days in order to allow the parasite an opportunity to develop. Afterwards some of the leeches were allowed to bite healthy animals while others were either fed or injected to normal guinea pigs. As shown in Table 5, the results of the experiments were negative. The microscopic examination of the leeches for developmental forms of trypanosomes was likewise negative.

TABLE 4.—*Direct transmission experiments with Hæmadipsa zeylanica.*

Interval between bites from infected to healthy animal.		Number of leeches applied.	Healthy animal exposed to leeches.	Fate of animal exposed.
H.	*m.*			
0	2	2	Guinea pig 4	Negative; kept under observation for 32 days.
0	3	4	Mouse 7	Negative; kept under observation for 28 days.
0	5	4	Rat 4	Negative; kept under observation for 32 days.
0	5	2	Mouse 2	Positive on 5th day; died on 8th day.
0	30	3	Mouse 15	Positive on 7th day; died on 10th day.
0	25	3	Mouse 16	Negative; kept under observation for 20 days.
0	25	3	Mouse 17	Negative; kept under observation for 25 days.
1	35	6	Guinea pig 7	Do.
1	45	4	Mouse 19	Negative; kept under observation for 24 days.
0	30	6	Guinea pig 6	Negative; kept under observation for 28 days.
0	10	4	Guinea pig 9	Do.
0	13	2	Mouse 20	Do.
0	8	10	Guinea pig 10	Positive on 8th day; died on 23d day.
0	5	3	Guinea pig 11	Negative; kept under observation for 32 days.
0	10	3	Guinea pig 12	Do.
0	15	[a] 6	Guinea pig 14	Positive on 10th day; died on 52th day.
0	10	[a] 6	Guinea pig 15	Negative; kept under observation for 28 days.
0	15	2	Guinea pig 16	Do.
0	10	[b] 9	Guinea pig 17	Negative; kept under observation for 34 days.
0	7	[b] 9	Guinea pig 18	Positive on 9th day; died on 25th day.

[a] Two leeches applied on the same animal for three consecutive days.

[b] Three leeches applied on the same animal for three consecutive days.

Transmission experiments with Hæmadipsa zeylanica.—As shown in Table 4, five of the twenty experiments on the mechanical or direct transmission of surra through the bite of the land leech yielded positive results. These constitute the first experimental data available on the transmission of a mammalian trypanosome by an annelid.

As shown in Table 5, attempts to transmit the disease indirectly gave uniformly negative results, indicating that, as in the carabao leech, the surra trypanosome does not undergo cyclic development in *Hæmadipsa zeylanica.* The land leeches used were found to harbor a species of trypanosome or what appeared to be its developmental stages, but these have no relation to *T. evansi* since they were not infective to mice, rats, and guinea pigs and, besides, were also found in leeches not fed on infected blood.

TABLE 5.—*Indirect transmission experiments.*

Leeches used.		Interval after leeches sucked infected blood.	Flagellated Protozoa found in leeches.	Method of infecting healthy animal.	Healthy animals exposed.	Result of experiment.
Kind.	Number.					
		Days.				
Hirudinaria	1	29	None	Saline suspension injected subcutaneously.	Two guinea pigs	Negative.
Do	1	44	do	Fed by mouth	do	Do.
Do	2	44	do	Allowed to bite	One guinea pig	Do.
Hæmadipsa	1	25	Trypanosomes and crithidia.	Saline suspension injected subcutaneously.	Two mice	Do.
Do	3	32	do	do	Three mice	Do.
Do	2	32	do	Allowed to bite	One guinea pig	Do.
Do	3	44	do	do	do	Do.
Do	7	44	do	Fed by mouth	Two guinea pigs	Do.

DISCUSSION OF RESULTS

No explanation can be given at the present time for the negative results obtained in the experimental transmission of surra by the use of the water leech, *Hirudinaria manillensis*. As far as I am aware, the question of why certain kinds of blood-sucking insects, such as *Tabanus*, can play the rôle of transmitters of the surra trypanosome while other kinds of blood-sucking flies, such as *Lyperosia*, seem unable to do so, has also not been explained. [Consult Kelser's (1927) summary of the literature and experiments on surra transmission by blood-sucking arthropods.] One thing seems apparent, however, and that is the nonoccurrence of a regurgitation process of the gut contents of this and also of the land leech during the act of biting, for otherwise the results of the transmission experiments would not have been so uncertain (see also below).

Evidence that the infection was conveyed in the contaminated proboscis of the land leech.—By allowing horse flies, which had previously been allowed to suck on a surra animal, to bite successively three healthy horses, of which only the first one subsequently developed the disease, Mitzmain (1914) was able to prove that the infection is conveyed in the proboscis of the flies and "concluded that the biting of *Tabanus striatus* is innocuous in infecting more than one horse as a result of a previous contamination." Similar experiments were performed by substituting land leeches for horse flies and the results obtained were identical with that of Mitzmain. It appears, therefore, that the transmission of experimental surra by *Hæmadipsa zeylanica* is effected through the insertion of its soiled proboscis and that the leech, like the *Tabanus*, is capable of infecting only one animal as a result of a previous contamination.

Number of land leeches applied on individual animals.—As shown in Table 4, the number of leeches used in the experiments on mice do not appear to have decided the outcome of any particular experiment. But on guinea pigs the results were more certain when a larger number of leeches were transferred from an infected to a healthy animal. This is to be expected, for under equal circumstances the greater the number of leeches biting, the greater will be the probabilities of their transmitting the infection. Rogers (1901) in India, working with horse flies, also obtained more uniformly positive results by using a larger number of flies in each of his transmission experiments.

Intervals after biting infected hosts.—With insects like *Tabanus*, the question of the length of time that they can remain infective after soiling their probosces with the blood of an infected animal is not so very important, because they can fly from one animal to another with great facility. With leeches, however, which do not have the same facility of movement, this is a most important question in a consideration of their probable rôle in nature as transmitters of surra. In the experiments recorded in Table 4, the intervals between the bites in four of the cases in which positive results were obtained were from five to ten minutes, while in one experiment it was thirty minutes. Whether or not they could have remained infective after a longer interval than thirty minutes, the results of the experiments do not show. It is believed, however, that the intervals mentioned are sufficiently extended to allow these annelids, in their natural environment, to apply themselves to a fresh victim if they happened to get dislodged from a first one.

POSSIBLE IMPORTANCE OF THE LAND LEECH IN THE MECHANICAL TRANSMISSION OF SURRA IN NATURE

Having shown that experimental surra can be transmitted mechanically by *Hæmadipsa zeylanica,* the following questions now present themselves: Does the land leech indulge in interrupted feedings in its natural environment? To what possible extent can the land leech be a factor in the incidence of surra? As to the first question, it is reasonable to suppose that any animal, like a leech or even a *Tabanus,* if left undisturbed, would prefer to play safe and finish a full meal, once it had started, than to feed a little from one place and then from another, thereby running the risk of not obtaining a sufficient amount. It so happens in the particular case of blood-sucking flies that they are naturally subject to very frequent interruptions during the procurement of their food, due to the efforts of their victims to drive them away, and by going from one animal to another they incidentally serve to spread infections. Even leeches with their tenacity to cling to the bodies of their victims may also be subject to interruptions for they may be shaken or brushed from an animal's body by branches of trees and other vegetation under or through which the animal may be passing. If the annelid has just started to suck when it is thus thrown off, there is no reason why it should not complete its meal on another available animal. Experimentally, at least, it has been shown that it will do so.

Regarding the extent to which the land leech may possibly be involved in the natural transmission of surra, it has been mentioned that this annelid is confined in its distribution to moist mountainous places seldom frequented by horses, so that it would seem that it can only be of very small or even negligible importance in the propagation of the disease among domesticated animals. In certain instances, however, it may play a decidedly important rôle. It is known that there are animals, such as pigs [according to Baldrey (1910)], carabaos, and cattle that live in mountainous places, where *Hæmadipsa zeylanica* prevails, and which can harbor the surra trypanosome in the capacity of reservoirs. The land leech may be involved in the mechanical transmission of the parasite from one to another of these animals, as well as from any one of them to an occasional mountain visitor, such as a hunting dog or a mounted horse. The latter, if infected, returns to the lowlands and may then spread the infection to other animals through blood-sucking flies.

SUMMARY AND CONCLUSIONS

The suspected rôle of leeches in the transmission of mammalian trypanosomes has so far not been demonstrated experimentally. It was felt, judging from the scarcity of the available literature, that the question has not yet been thoroughly investigated and that further observations are necessary. For this reason it was decided to inquire into the behavior of a laboratory strain of *Trypanosoma evansi* of equine surra in the bodies of leeches and into the possibility of its being transmitted by them.

Two species of leeches were used in the experiments; namely, the water leech, *Hirudinaria manillensis* (Lesson), and the land leech, *Hæmadipsa zeylanica* (Moquin-Tandon).

By injecting the blood of leeches that had sucked infected animals into mice, at different intervals, it was determined that the surra parasite cannot survive long in the digestive tract of the water leech; it was infective up to one hour and twenty minutes after biting, but not after two hours and ten minutes. In the digestive tract of *Hæmadipsa zeylanica* it remained viable up to four hours and fifteen minutes, but not five hours and forty-five minutes after feeding.

By means of interrupted-feeding experiments it was also determined that the water leech is unable to transmit surra either directly or indirectly. On the other hand positive results were obtained by the use of *Hæmadipsa zeylanica.*

The evidence is that the transmission of the disease by the land leech was direct or mechanical, the infection being conveyed in the proboscis as the result of a previous contamination and retained for as long as thirty minutes.

Attempts to infect laboratory animals indirectly, by injecting or feeding them with the contents of land leeches that had been kept alive for as long as forty-four days after feeding on infected blood, yielded negative results. It may be concluded, therefore, that the surra trypanosome does not undergo cyclical development in the body of *Hæmadipsa zeylanica.*

Crithidial and trypanosomal flagellates were encountered in the digestive tract of some of the land leeches used in the transmission experiments, but they failed to infect when they were injected or fed to mice and guinea pigs. They were also found in leeches not fed on surra-infected blood, so that they probably represent parasites peculiar to the land leech or they are the developmental stages of a trypanosome other than *T. evansi.*

Taking for granted that the land leech can transmit surra in nature, as has been demonstrated experimentally, it would appear that it can only play a minor rôle as mechanical carrier during an outbreak of the disease due to its restricted distribution in places not usually frequented by domesticated animals. Its importance as a transmitter would be as an agent in the transfer of the parasite from some reservoir host that lives in the mountains, such as a pig or a carabao, to a chance animal, like a hunting dog or a horse ridden to the mountains. Any of the latter, if infected, or returning to the lowlands may spread the infection through the agency of blood-sucking flies.

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ILLUSTRATION

PLATE 1

FIG. 1. *Hirudinaria manillensis* (Lesson), ventral and dorsal views.
2. *Hæmadipsa zeylanica* (Moquin-Tandon), dorsal views.

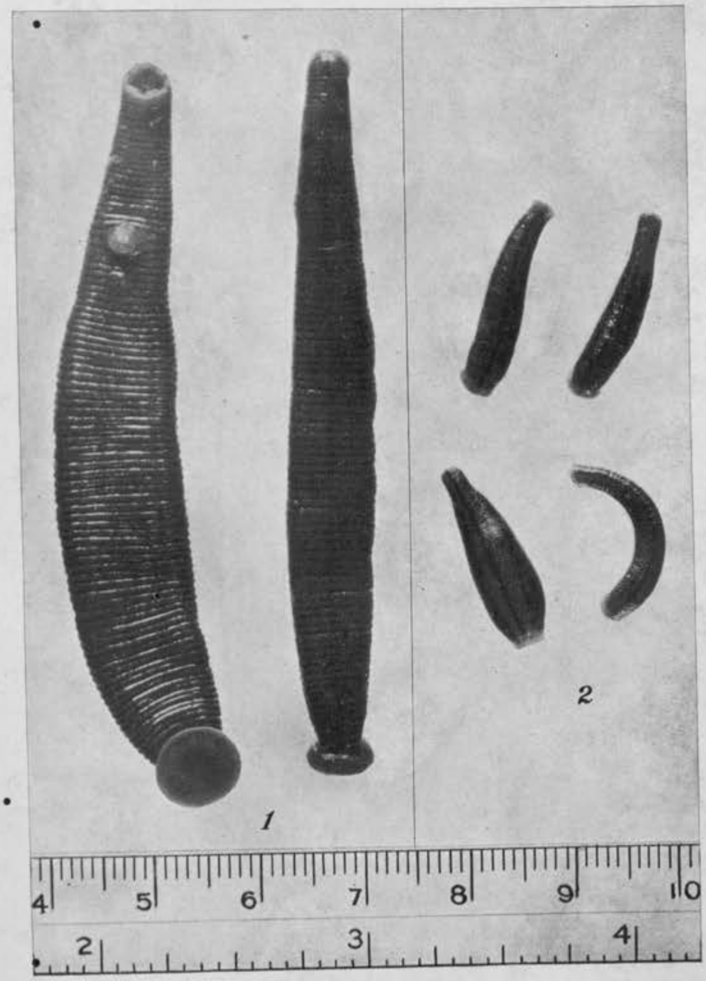

PLATE 1.